UNIVERSITY OF ALBERTA

DEVELOPMENT OF A BARLEY β-GLUCAN BEVERAGE WITH AND WITHOUT WHEY PROTEIN ISOLATE

by

CRAIG BANSEMA

©

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled ***DEVELOPMENT OF A BARLEY β-GLUCAN BEVERAGE WITH AND WITHOUT WHEY PROTEIN ISOLATE*** submitted by Craig Bansema in partial fulfillment of the requirements for the degree of Master of Science in Food Science and Technology.

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ABSTRACT

Mixed-linkage (1→3), (1→4)-β-D-glucan, a soluble fibre component of barley and oats, has demonstrated health benefits. Barley β-glucan functional properties and interactions with sucrose and whey protein isolate (WPI) as well as temperature and pH effects on sol rheology were investigated. Sucrose and WPI addition increased ($p \leq 0.05$) the viscosity of β-glucan sols. β-Glucan gum increased ($p \leq 0.05$) WPI heat stability, as determined by differential scanning calorimetry. β-Glucan-WPI phase separation behavior was also examined. β-Glucan was susceptible to acid-catalyzed hydrolysis at pH 3 with a 30 min heat treatment at 55, 75 and 95°C.

Functional beverages were developed incorporating barley β-glucan with and without WPI. Trained panel and consumer sensory evaluations of beverages were performed. Beverages were found to be acceptable by a consumer panel and were stable throughout 12 weeks of refrigerated storage. The successful formulation of barley β-glucan functional beverages represents only one potential application for this valuable ingredient.

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1. INTRODUCTION AND THESIS OBJECTIVES

There is growing research interest in barley and oat soluble fibre rich in β-glucan and milk whey proteins, both possessing beneficial nutritional and valuable functional properties, and the development of food and beverage products containing these ingredients show much potential. This research and development is further supported by the current consumer demand and market for health-promoting products. Industry has already responded and now produces a variety of foods and beverages "enhanced" with ingredients such as fibre, antioxidant vitamins and herbal extracts. However, products containing or enriched with barley β-glucan soluble fibre, other than the limited barley products, are not available. Whey and whey protein isolate have seen some applications in food and beverage production, however, its behavior and compatibility with other health-promoting ingredients, such as barley β-glucan, is unknown.

Alberta is a major producer of barley, accounting for 50% of Canada's production. Barley is an excellent source of β-glucan. Whey, a nutritious byproduct of the cheese industry, will be available as long as there is cheese production. It would, therefore, be advantageous to find applications for, and produce foods and beverages containing, these ingredients.

Many whey-based beverages have been produced, taking advantage of the excellent nutritional properties as well as health-promoting potential of whey or whey protein isolates. There has been some work done to determine the

functionality and behavior of whey and whey protein isolates in such products and their processing. However, these products have focused on whey components as the principal health-promoting ingredient. Formulation of functional foods containing other compounds in addition to whey proteins, and knowledge of the associated interactions, is limited. Understanding of interactions between whey proteins and other ingredients, such as barley β-glucan, will help in formulating foods and beverages with health promoting properties.

Research on the functional properties of barley β-glucan, especially pertaining to food and beverage applications, and its interactions with other food components, such as whey proteins, is limited. The influence of barley β-glucan on sensory attributes of products with and without added whey protein on product sensory attributes is unknown. The literature also lacks information on the stability of the barley β-glucan polysaccharide during processing treatments and shelf-life of a product. As a result, there are no food products developed with barley β-glucan extracts. However, barley β-glucan shows much potential as a functional and health-promoting ingredient. The combination of barley β-glucan and whey protein isolate in a beverage would result in a product offering the potential health benefits of both compounds.

Therefore, the objectives of this thesis are:

1. to examine the effects of pH/temperature treatments, sucrose- and whey protein isolate-β-glucan interactions on the functional properties of barley β-glucan sols,

2. to produce a barley β-glucan-based beverage and examine the sensory properties, consumer acceptability and shelf stability of the beverage, and
3. to produce a barley β-glucan/whey protein beverage and examine the sensory properties, consumer acceptability and shelf stability of the beverage.

2. LITERATURE REVIEW

2.1. BARLEY

Cultivated barley (*Hordeum vulgare*), referred to as barley hereafter throughout this thesis, is classified as one of the original ancient cereal grains consumed in many countries around the world throughout history. Barley has been recorded as being cultivated along the Nile River thousands of years ago, dating back to Egyptian times (Wendorf et al., 1979). More recently, however, with the increase in the consumption of baked products, such as breads and pastries, and pastas, human intake of barley has declined and that of wheat increased. Wheat has proven to be more suitable for baking applications when compared to barley (Bhatty, 1993c). Barley is currently used primarily for livestock feeds, and the remainder is utilized in the malting, brewing, and food production industries. Barley has recently been rediscovered as a nutritious food grain for the human diet and is expected to see some increase in food applications in the near future.

2.1.1. Production

Currently, barley is grown and harvested throughout the world. Barley ranks fourth in worldwide cereal grain production, following wheat, rice and corn (Nilan and Ullrich, 1993). In Canada, however, it ranks second highest for crop production, being exceeded only by wheat. During the 1992/93 to 1996/97 growing seasons, an average of 4,441,000 hectares were dedicated to barley in Canada. Canadian barley growers averaged 3.01 tonnes/hectare for a total

production of 13,357,000 tonnes of barley grain. An average of 9,287,400 tonnes was used domestically and 3,037,200 tonnes was exported (Alberta Agriculture, Food and Rural Development, 1999). In Canada, barley is grown primarily across the prairie provinces, Alberta, Saskatchewan and Manitoba. Alberta leads Canada in the production of barley, producing 50% of all barley grown in Canada (Statistics Canada, 1995). Alberta Agriculture, Food and Rural Development (1999) is predicting that the amount of grain exported will decrease and the amount of barley product exports will increase during the 1997-2000 period.

2.1.2. Current utilization

Only 5% of the barley produced in Canada is currently being utilized for direct human consumption. This includes products such as pot and pearled barley, and barley flour, which are available to consumers in the retail market. Human consumption of barley is expected to increase in the near future (Bhatty, 1986). Barley grain has been shown to be an excellent source of both soluble and insoluble fiber, which according to dietitians and health professionals, should be increased in diets to improve health (Newman and Newman, 1991, Oscarsson et al., 1996). The typical Canadian diet does not contain the 20-35 g of fiber per day, recommended by Health and Welfare Canada (1985). Barley, having a weaker, non-elastic protein network than that of wheat, does not form as good a gluten protein structure for the production of leavened baked products. Barley flour, however, would work well in the production of unleavened products, such as flat breads and pastas (Bhatty, 1993c) and has successfully been used to replace a portion of the wheat flour in bread (Hawrysh et al., 1996).

Approximately 10% of Canadian barley is further processed by the malting industry for use in foods and beverages (Bhatty, 1986). Barley malt syrup can be found on the ingredient lists of many food products, including breakfast cereals and baked products. Malt syrup is used to enhance the color and flavor of foods in such applications (Bamforth and Barclay, 1993).

Malted barley is, however, primarily used by the brewing industry for the production of beer and also in the production of distilled spirits. Barley is an indispensable ingredient functioning as the nutritional energy source of brewer's yeast (*Saccharomyces cerevisiae*) for the production of ethanol. Malting varieties of barley are grown solely because of their excellent properties for malting and brewing applications (Bamforth and Barclay, 1993).

The remaining 85% of barley cultivated in Canada is utilized in animal feed for the beef, pork and poultry industries (Bhatty, 1986). Barley is a rich source of the digestible carbohydrates and proteins required for raising livestock for food. High-lysine varieties of barley (lysine is the limiting amino acid in livestock feeds) are available and are used in the development of high-lysine feed barley (Bhatty, 1993b).

2.1.3. Composition

Barley is a typical cereal grain composed primarily of starch, protein, fiber, lipids, and minerals. The basic composition of barley is outlined in Table 2.1. The exact composition of barley will, however, vary depending on the variety chosen and growing conditions the barley is exposed to. Variables such as soil composition, moisture, temperature and amount of sunlight, which are difficult or

Table 2.1. Typical chemical composition of barley grain.[1]

Component		% (w/w) (dry wt. basis)
Carbohydrates		78-83
	Starch	63-65
	Sucrose	1-2
	Other Sugars	1
	Water-soluble polysaccharides	1-1.5
	Alkali-soluble polysaccharides	8-10
	Cellulose	4-5
Lipids		2-3
Protein		10-12
	Albumins and Globulins	3.5
	Hordeins[2]	3-4
	Glutelins	3-4
Nucleic Acids		0.2-0.3
Minerals		2
Other		5-6

[1] Adapted from MacGregor and Fincher (1993).
[2] Prolamin fraction known as hordeins in barley (Shewry, 1993).

impossible to control, can produce location and seasonal variation in the grain composition of barley crops (Duffus and Cochrane, 1993).

The composition of barley demonstrates its value as a food source. Barley is a rich source of insoluble and soluble dietary fibre, containing 11-21% depending on the variety selected (Newman and Newman, 1991; Oscarsson et al., 1996). Its addition to the diet would help meet the daily fibre intake recommended by dietitians and health professionals. The starch portion of the grain is a good source of digestible carbohydrate, necessary for energy. Barley is also a good source of protein, typically containing 10-12% in the whole grain. Barley contains more of the essential amino acid lysine, typically low in cereal grains, when compared to wheat (Chung and Pomeranz, 1985). Barley proteins can be grouped as storage and non-storage proteins. Storage proteins include the prolamins (hordeins in barley) and globulins, as defined by Osborne protein classification, which are utilized during germination to provide nutrients for the developing seedling (Shewry, 1993). The non-storage albumins and glutelins, also Osborne protein classes, serve seed structural and metabolic functions (e.g. enzymes).

Barley lipids contain tocopherols and tocotrienols (tocols), which have vitamin E and antioxidant activity (Morrison, 1993). Barley contains 42-80 mg of tocols per kilogram, depending on variety and growing conditions (Peterson and Qureshi, 1993). All eight tocol isomers are found in measurable quantities in barley, and are of favorable distribution of the most biologically active isomers, making it unique among cereal grains (Morrison, 1993; Peterson and Qureshi, 1993). Despite these benefits, human food applications for barley remain limited.

2.2. β-GLUCAN

β-Glucan is a soluble fiber component found primarily in the endosperm cell walls of cereal grains, especially those of barley and oats. β-Glucan has recently been found to be a valuable component of barley, possessing many nutritional and functional properties. This has encouraged much research into the extraction and concentration, characterization, and application of cereal β-glucans.

β-Glucan, until recently, was viewed as an undesirable component of barley. This polysaccharide, typically ranging from 4-7% in barley, increases the viscosity of water-flour mixes, making barley flour difficult to work with for the production of baked goods (Bhatty, 1986). β-Glucan is also responsible for plugging filters during the brewing process and causing haze formation in the final beer product. It also decreases the efficiency of poultry feed by inhibiting nutrient absorption and causing animals to produce sticky feces, creating waste disposal problems (MacGregor and Fincher, 1993). For these reasons, many barley growers selectively grow barley varieties lower in β-glucan to minimize production problems and economic losses in those industries. β-Glucanases have been added to barley mixes to solve β-glucan induced problems, but at additional cost to producers and consumers. β-Glucanases easily hydrolyze the β-glucan polysaccharide, thus decreasing the viscosity of barley containing mixes that may be utilized for feed or food (Bhatty, 1986). β-Glucan is now, however, being viewed as a very valuable nutritional component and its presence in the barley grain targeted for food use is beneficial.

2.2.1. Occurrence in barley and other cereal grains

β-Glucan is found at a concentration ranging from 2-10% in the whole barley grain (Lee et al., 1997). However, some varieties, such as Prowashunupana (ConAgra Specialty Grain Product Co., USA), may contain up to 15% β-glucan. Such varieties are, therefore, preferred for the isolation of β-glucan, and will produce the greatest extract yield. Typically, waxy and hulless varieties of barley, such as Prowashunupana, have a higher concentration of β-glucan (Bhatty, 1999). Oats also contain a higher amount of β-glucan (3-7%) than most other cereal grains. β-Glucan is also found in the cell walls of wheat, rye, maize, rice, sorghum and millet (Lee et al., 1997).

2.2.2. β-Glucan structure

Mixed-linkage (1→3), (1→4)-β-D-glucan (β-glucan) is an unbranched polysaccharide composed of (1→4)-β-linked glucose (as found in cellulose), with (1→3)-β-linkages separating the (1→4)-β-linked cellotriosyl and cellotetraosyl units (Fig. 2.1). The cellotriosyl and cellotetraosyl units are randomly distributed throughout the polysaccharide. The structure of β-glucan was determined using magnetic resonance spectroscopy techniques by Wood et al. (1994).

β-Glucan structure may also be simply represented by the following, where 3 and 4 represent the β-(1→3) and β-(1→4) linkages and G represents the glucose subunits of the polysaccharide. Bracketed units of 3 glucose (G), represent cellotriosyl units and units of 4 glucose (G), represent cellotetraosyl units (MacGregor and Fincher, 1993).

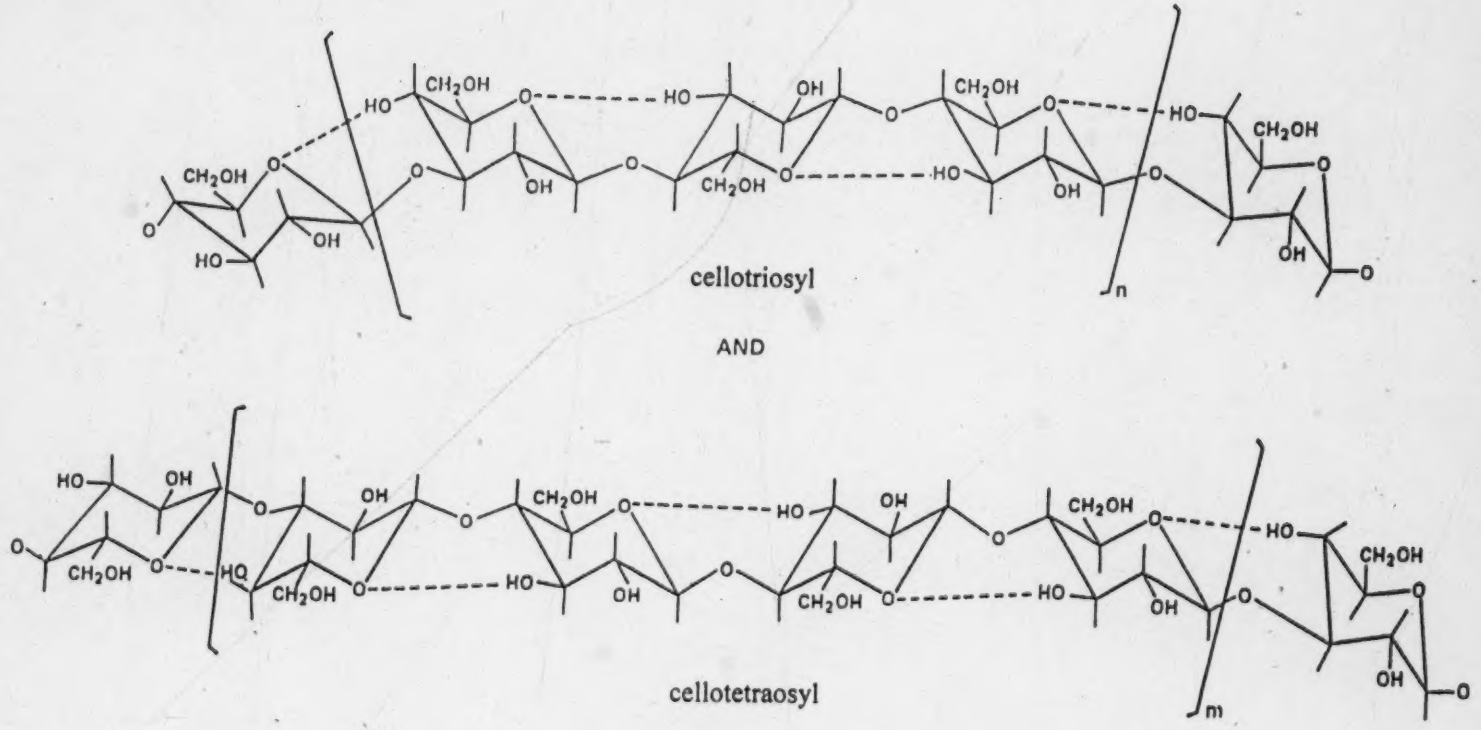
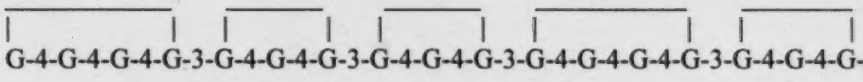


Figure 2.1. β-Glucan structure showing cellotriosyl and cellotetraosyl units of (1→4)-β-linkages separated by (1→3)-β-linkages. Adapted from Wood (1984).



Wood (1994) reported that the structures of barley and oat β-glucan are distinctly different. Barley β-glucan was found to contain close to one quarter β-(1→3) linked units, whereas, oat β-glucan contained approximately one third. The oat β-glucan structure, therefore, contains more β-(1→3) linkages than barley β-glucan.

The β-(1→3) linkage produces irregularities among the linear β-(1→4) linked backbone making the polymer water soluble, as compared to cellulose, which is composed solely of β-(1→4) linked glucose subunits and is completely insoluble in water. The irregularities in the polysaccharide structure allow for water to form hydrogen bonds with the hydroxyl groups found extending from the glucose subunits. This interaction and formation of hydrogen bonds result in the solubility of the polysaccharide.

2.2.3. Extraction of β-glucan

Barley and oats, being rich sources of β-glucan, have been used as starting material for the extraction and purification of β-glucan. β-Glucan has been extracted and concentrated from cereals using dry milling and sieving (Knuckles et al., 1992; Sunberg and Aman, 1994), dry milling and air classification (Wu et al., 1994) or alkali extraction procedures (Wood et al., 1989; Bhatty, 1993a; Dawkins and Nnanna, 1993; Saulnier et al., 1994; Bhatty, 1995; Temelli, 1997). Using the dry milling and sieving or air classification techniques, extracts containing 8-38% β-glucan have been produced. Solvent extraction, however, has

yielded gum extracts containing 51-89% β-glucan.

Burkus and Temelli (1998) have shown that extraction conditions, such as pH and temperature, greatly affect the viscosity of sols prepared with β-glucan concentrates. By controlling extraction conditions, extracts of desired viscosity, either high- or low-viscosity gums, can be produced to suit the desired application. If a higher concentration of β-glucan is desired in a product, low-viscosity extracts may be utilized with less change in functional properties, such as viscosity, than if a high-viscosity extract is used (Burkus, 1996).

β-Glucan gum may find application in foods. β-Glucan may be used to increase a product's soluble fibre content, improving its nutritional value. There is also the potential to label such products with health specific claims. Barley β-glucan addition will also result in alterations of a food's functional properties, such as viscosity. Therefore, examination of β-glucan's functional properties and its interaction with other ingredients, and testing of potential food product formulations, are required for the production of nutritionally enhanced products that still possess consumer appeal.

2.2.4. Functional properties of β-glucan

The addition of β-glucan to water results in the formation of a viscous hydrocolloid sol (Autio et al., 1987; Dawkins and Nnanna, 1995; Burkus, 1996). The polysaccharide's hydroxyl groups, extending from the glucose units of the polymer (Fig. 2.1), are available to form hydrogen bonds with water. This association between the polysaccharide and water makes the polymer water-soluble. There is also some degree of self-association between separate β-glucan

chains by the formation of hydrogen bridges, creating fringed micelles. These micelle structures, precursors to gel formation, can reach molecular weights of up to 50,000,000 D (Linemann and Kruger, 1998). The presence of polymers in solution creates a random network, which increases the internal friction within the solution. This results in an inhibition to internal flow and thus increase the solution viscosity (Glicksman, 1982).

As the concentration of β-glucan rich gum in a sol increases, sol viscosity will increase (Fig. 2.2). Rheological behavior will also change with concentration. At lower concentrations of β-glucan, sols may be described as demonstrating Newtonian behavior. The viscosity of a Newtonian sol will remain constant regardless of shear rate applied. However, at higher concentrations of β-glucan gum, pseudoplasticity may be observed. Pseudoplasticity is the shear thinning or viscosity decrease that occurs as shear rate increases (Glicksman, 1982; Dawkins and Nnanna, 1995).

Temperature will also greatly affect the viscosity of a β-glucan sol. As temperature increases, viscosity decreases and as temperature decreases viscosity increases. The sol's rheological behavior also changes with temperature. The sol may be pseudoplastic at lower temperatures, while exhibiting Newtonian behavior at higher temperatures (Autio et al., 1987; Dawkins and Nnanna, 1995).

Viscosity increased with the addition of sucrose to oat β-glucan sols (Dawkins and Nnanna, 1995). This was observed at sucrose levels of 20-45%, however, at 65% sucrose the solution viscosity was reported to decrease for oat β-glucan sols (Fig. 2.3). It is hypothesized that at high sucrose levels, water is taken

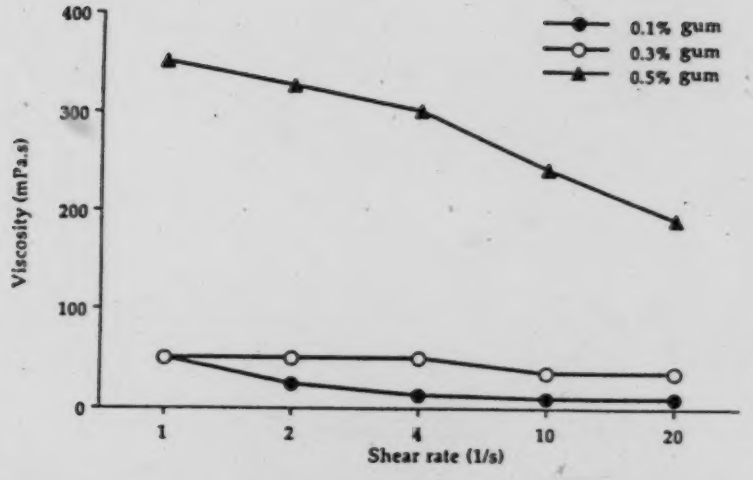


Figure 2.2. The effect of oat β-glucan gum (gum, OG) concentration on sol viscosity at 25°C and neutral pH. Reprinted from Food Hydrocolloids, Vol. 9, No. 1, Dawkins and Nnanna, Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties, pp 1-7, Copyright (1995), with permission from Elsevier Science.

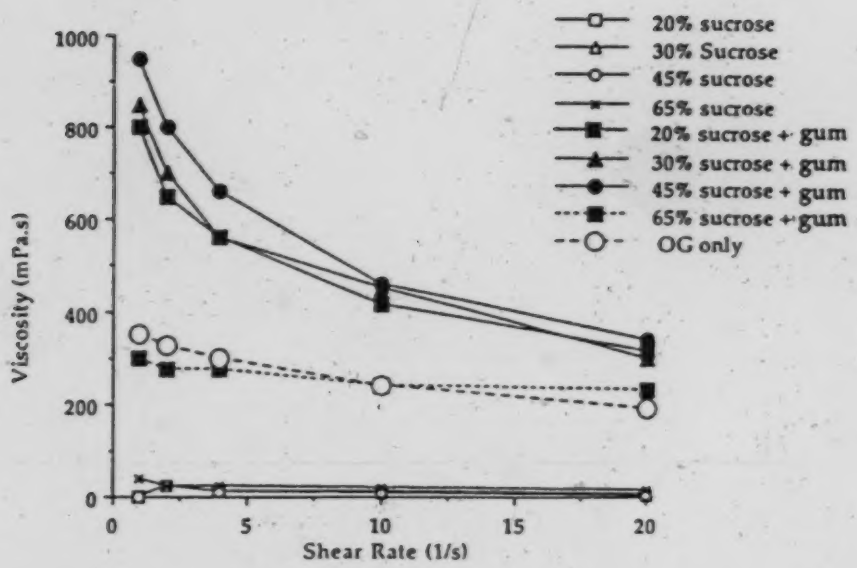


Figure 2.3. The effect of sucrose on viscosity of 0.5% oat β-glucan gum (gum, OG) sols at 25°C. Reprinted from Food Hydrocolloids, Vol. 9, No. 1, Dawkins and Nnanna, Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties, pp 1-7, Copyright (1995), with permission from Elsevier Science.

away from the polysaccharide. The competition between β-glucan and sucrose for water results in decreased hydration of the polysaccharide. The expansion of the β-glucan polymer is, therefore, decreased, resulting in a decreased sol viscosity.

The β-glucan polymer, being nonionic and neutral, is not affected by solution pH, therefore, viscosity is independent of pH (Fig. 2.4). This makes cereal β-glucans useful in various foods of different pH (Dawkins and Nnanna, 1995).

At low concentrations, β-glucan may be used as a thickener in food products. At higher concentrations, β-glucan may be used to form a gel (Burkus and Temelli, 1999a). Gelation occurs when association and/or cross linking of long polymer chains, such as those of β-glucan, forms a three dimensional network, trapping the water it is dissolved in. This forms a rigid structure referred to as a gel (Glicksman, 1982). β-Glucan, like starch, is a non-ionic polymer, it therefore does not require a specific pH or the presence of ions to form a gel (Dawkins and Nnanna, 1995; Burkus, 1996).

β-Glucan was also shown to aid in the stabilization of foams and emulsions (Burkus and Temelli, 1999b). As viscosity increases, the stability of an emulsion or foam increases. This is demonstrated by Stokes' law,

$$V = \frac{2gdr^2}{9n} \tag{2.1}$$

where V is the equilibrium velocity of phase separation, g is the gravity constant, d is the density difference between the phases, n is the viscosity in the region around the droplets and r is equivalent radius of the droplets (Hicks, 1990). The

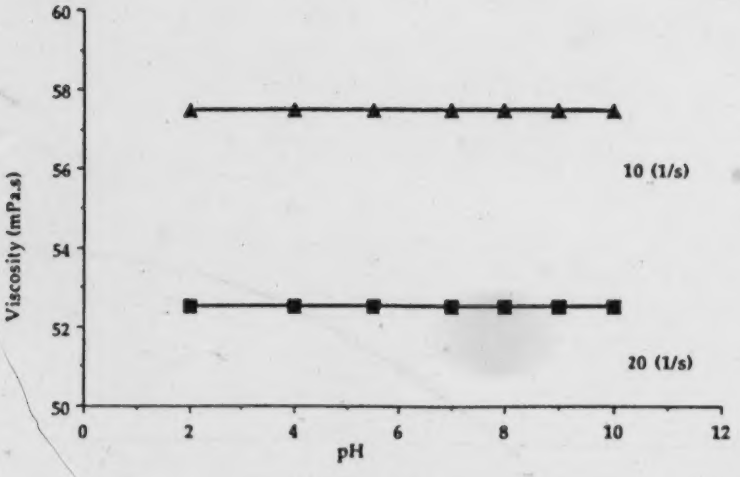


Figure 2.4. Viscosity of a 0.3% oat β-glucan gum sol as a function of pH. Reprinted from Food Hydrocolloids, Vol. 9, No. 1, Dawkins and Nnanna, Studies on oat gum [(1→3, 1→4)-β-D-glucan]: composition, molecular weight estimation and rheological properties, pp 1-7, Copyright (1995), with permission from Elsevier Science.

increase in viscosity will inhibit the separation of phases, therefore increasing emulsion stability. β-Glucan may also be used as a stabilizer to suspend particles in a solution, eg. spices in a salad dressing. The suspension stability of particulates in this type of system is also described by Stokes' Law (eq. 2.1).

β-Glucan may, therefore, be used in various applications where other thickeners, stabilizers, or gelling agents such as pectin, guar, carrageenan, and xanthan gum have been used. β-Glucan may be used to alter the viscosity of beverages, where pectin is traditionally used or potentially replace gelatin in jelly type desserts. The addition of barley β-glucan will also enhance a product's nutritional quality by increasing its soluble fiber content. Although β-glucan's potential functionality indicates it may be a versatile ingredient, there are no food or beverage products incorporating β-glucan rich isolates.

2.2.5. Health promoting properties of β-glucan

Along with beneficial functional properties, β-glucan was also shown to have many health-promoting properties (Klopfenstein, 1988; Wood and Beer, 1998). The hypocholesterolemic effects of β-glucan, from barley and oats, have been examined in both animal (Newman et al., 1992; Wang et al., 1992; Kahlon et al., 1993, Wang et al., 1997; Wood and Beer, 1998; Bell et al., 1999) and human studies (Klopfenstein and Hoseney, 1987; Casterline et al., 1997; Wood and Beer, 1998; Bell et al., 1999). Newman et al. (1989) examined the effects of moderate levels of dietary cereal β-glucans and β-glucan's ability to lower serum cholesterol. Their results showed a general decrease in total and LDL-cholesterol (Table 2.2) with varied response among individual participants. Individuals with

Table 2.2. Changes in serum lipids (mean ± standard error) of adults after consuming oat or barley foods (mg/dl). Adapted from Newman et al. (1989).

Serum Lipid Constituent	Oat Group			Barley Group		
	Initial	Mid[a]	Final[b]	Initial	Mid[a]	Final[b]
Total cholesterol	248 ± 10.3	235 ± 11.2	236 ± 10.5	256 ± 17.4	248 ± 16.9	244 ± 15.7
LDL-cholesterol	157 ± 10.0	151 ± 8.9	146 ± 9.0	173 ± 14.7	158 ± 18.2	149 ± 16.0
HDL-cholesterol	63 ± 6.5	52 ± 6.3	57 ± 6.2	49 ± 3.9	53 ± 4.1	54 ± 3.9
LDL/HDL	2.73 ± 0.3	2.92 ± 0.3	2.69 ± 0.2	3.66 ± 0.3	3.29 ± 0.3	3.21 ± 0.4
Triglyceride	139 ± 33.7	164 ± 24.8	161 ± 32.3	171 ± 43.6	194 ± 56.3	182 ± 40.1

[a] Mid = 3 weeks.
[b] Final = 6 weeks.

elevated initial serum cholesterol levels showed more significant decreases than those with normal or low initial levels.

The mechanism of β-glucan's cholesterol lowering action has been hypothesized to be the result of the increase in viscosity or thickening of the contents of the intestine. The thickening action of β-glucan binds up cholesterol and cholesterol metabolites (i.e. bile salts), preventing absorption or re-absorption, respectively, into the blood (Klopfenstein and Hoseney, 1987; Newman et al., 1992; Wang et al., 1992; Kahlon et al., 1993, Wang et al., 1997; Wood and Beer, 1998). The fermentation of β-glucan by the microflora in the colon results in the production of short chain fatty acids (SCFA), such as acetic, propionic and butyric acid. SCFA, when reabsorbed, have been shown to have hypocholesterolemic properties as well (Casterline et al., 1997; Wood and Beer, 1998; Bell et al., 1999).

β-Glucan has also been demonstrated to aid in the control of blood glucose levels. When soluble fiber consumption is increased, the postprandial rise in blood insulin is decreased (Klopfenstein, 1988; Wood et al., 1990; Braaten et al.,

1991). The addition of β-glucan rich grains or β-glucan concentrates to enrich the soluble fibre content of foods could, therefore, help individuals with diabetes.

β-Glucan shows much potential for incorporation into the formulation of foods and beverages that may have benefits, beyond basic nutritional effects, and is therefore, anticipated to appear on ingredient labels. Currently, no food products, other than oat and barley products, are being produced which are enriched with β-glucan.

2.3. WHEY AND WHEY PROTEINS

Whey is the term used to describe the greenish fluid that separates from clotted milk when producing cheese or during the recovery of casein, the principal milk protein. Separation of the two groups of milk proteins, casein and whey proteins, may be accomplished by either, acid or enzymatic coagulation (Jelen, 1991). Traditionally, whey has been viewed as a waste product of the cheese industry and was poured into streams and municipal sewage systems. However, because of rising disposal costs, research has emphasized the recovery of whey and its food product applications. It has been proven to be a valuable ingredient and it is currently being used for its nutritional and functional properties (Holsinger et al., 1974; Cooper et al., 1977; Jelen, 1983; Beukema and Jelen, 1990; Mandal et al., 1997; Zydney, 1998).

2.3.1. Composition of whey

The composition of sweet and acid whey, as obtained by enzyme or acid coagulation of casein, respectively, is outlined in Table 2.3. The raw, liquid whey

Table 2.3. Composition (g/L) of sweet and acid whey.[1]

Component	Sweet Whey (pH 5.9-6.4)	Acid Whey (pH 4.6-4.8)
Total solids	63.0 – 70.0	63.0 – 70.0
Protein (N x 6.37)	6.0-8.0	6.0-7.0
Lactose	46.0-52.0	44.0-46.0
Fat	0.2-1.0	0.1-0.5
Calcium	0.4-0.6	1.2-1.6
Magnesium	0.08	0.11
Phosphate	1.0-3.0	2.0-4.5
Citrate	1.2-1.7	0.2-1.0
Lactate	2.0	6.4
Sodium	0.4-0.5	0.4-0.5
Potassium	1.4-1.6	1.4-1.6
Chloride	1.0-1.2	1.0-1.2

[1] From Jelen (1991).

contains many milk proteins including α-lactalbumin, β-lactoglobulin, serum albumin, immunoglobulins, lactoferrin, and lactoperoxidase. The protein composition and properties of whey protein fractions are displayed in Table 2.4.

2.3.2. Isolation of whey and whey proteins

To separate whey from milk, casein, the principal milk protein, must be coagulated. For acid coagulation of casein to occur the pH of the milk is lowered to 4.5-4.8 either by the addition of acid (e.g. acetic, phosphoric, hydrochloric, lactic, or citric acid) or by fermentation due to the growth of lactic acid producing microorganisms added to milk. The addition of acid is used in the industrial separation of casein for the production of caseinates. Fermentation is employed for the manufacture of fresh cheeses such as quarg or cottage cheese. The whey produced by these methods is referred to as acid whey (Jelen, 1991).

Sweet whey is produced when coagulation is accomplished using enzymatic methods through the use of rennet. Sweet whey comes from the production of hard and semi-hard cheeses such as Cheddar, Swiss and Gouda. This enzymatic process may occur at pH 6.0 or higher, therefore, the lactic acid content of sweet whey is low. The pH of sweet whey will, however, quickly drop if pasteurization is not used to control the growth of lactic acid bacteria present in the separated whey (Jelen, 1991).

Whey is obtained in a ratio of 9:1 (whey:cheese) from milk during cheese making. Therefore, for every 10 L of milk used for cheese production, 9 L of whey will be produced (Jelen, 1983). Raw whey contains 93-94% water, 4.5-5.0% lactose, 0.4-0.7% minerals and 0.6-0.8% proteins. Therefore, methods are often

Table 2.4. Whey protein composition and properties of whey protein fractions.[1]

Protein	Concentration, g/L whey	Isoelectric pH	Denaturation Temperature, °C	Heat Precipitation Stability
α-lactalbumin	1.2	4.5-4.8	61	Quite Stable
β-lactoglobulin	2.7	5.2	82	Labile
Serum albumin	0.4	4.7-4.9	66	Very labile
Immunoglobulins	0.65	5.5-8.3	72	Extremely labile
Lactoferrin	0.1	9.0		
Lactoperoxidase	0.02	9.5		
Other (casein, glycomacropeptide)	0.1	-		

[1] From Jelen (1991) and Zydney (1998).

employed to concentrate the desired product, the whey proteins. This further processing may be accomplished using such processes as reverse osmosis (RO), ultrafiltration (UF), microfiltration (MF), electrodialysis (ED), ion exchange (IE) and/or lactose hydrolysis (LH) (Jelen, 1991; Zydney, 1998).

UF is, however, most often used to concentrate the whey proteins. The UF retentate contains the whey proteins, whereas the UF permeate contains lactose and minerals. Diafiltration, repeated rinsing of the UF retentate with water, is also used to remove more of the residual lactose and minerals. The retentate is then dried at low temperatures, so that the proteins are not denatured. Such further concentration is used to produce whey protein concentrates (WPC), 35-80% protein, and whey protein isolates (WPI), >90% protein. WPC and WPI may be used as food and beverage ingredients for both their functional and nutritional properties (Clark, 1991; Jelen, 1991; Zydney, 1998).

2.3.3. Functional properties of whey proteins

WPC and WPI produced by the concentration of proteins from raw whey, have unique functional properties. These powders are water-soluble and form a stable solution when mixed with water. The addition of WPC or WPI to water results in an increase in viscosity or thickening and an alteration in solution rheological properties (Zydney, 1998). With an increase in temperature, the whey proteins begin to aggregate and the solution viscosity increases. Whey protein gels can be produced by heating WPI solutions to temperatures high enough to facilitate complete protein aggregation and gelation (Cooper et al., 1977; Walkenstrom et al., 1998).

Upon the application of a high shear rate, or whipping action, to a whey protein solution, air is captured within the mixture and a foam is produced (Zydney, 1998). The stability of this foam may be increased with the addition of a polysaccharide gum, such as barley β-glucan, prior to whipping (Burkus and Temelli, 1999b). Therefore, WPC and WPI may be used as foaming agents or in the production of meringue type desserts.

A change in color to a milkier, creamier appearance also occurs with WPC and WPI addition to a sol or solution. The degree of this color change is, however, dependent upon such things as sucrose and salt concentrations (Cooper et al., 1977). The whey also has a specific flavor, which will be carried over to the product that it is added to. This flavor was found to be acceptable in consumer evaluations of whey protein beverages (Holsinger et al., 1974). WPC and WPI have also been used for their emulsifying and water holding capacity. Such functional properties may be taken advantage of in foods and, WPC and WPI may be used to formulate food products with desirable functional and nutritional properties.

The combination of a polysaccharide sol and protein solution creates a complex set of interactions within the polysaccharide-protein-water system. Interactions between polysaccharide and protein biopolymers have been reviewed extensively (Tolstoguzov, 1991; Samant et al., 1993; Syrbe et al., 1998). The interaction of polysaccharides and proteins results in either of three scenarios: co-solubility, complexing or incompatibility. Co-solubility is the least typical outcome of the combination of these ingredients. More often, attraction or

repulsion between the biopolymers results in complexing or incompatibility, respectively. If these biopolymers are attracted to each other, a soluble or insoluble complex can form and a single- or a two-phase system is produced, respectively. Incompatibility also can result in phase separation if the biopolymer concentration is above the phase separation threshold but complexing is inhibited. Each phase of this two-phase system will be rich in one of the biopolymers (e.g. polysaccharide), with a smaller amount of the other (e.g. protein) and the other phase will be composed of the reverse ratio of biopolymers (e.g. protein rich with a smaller amount of polysaccharide). Incompatibility favors the self-association of macromolecules. If, however, the concentration of biopolymers is below the phase separation threshold, a single-phase system will be produced.

Interactions between neutral D-glucan polysaccharides (amylopectin, glycogen and dextran), similar in composition to β-glucan, and gelatin were investigated by Grinberg and Tostoguzov (1972). It was found that gelatin and D-glucans were thermodynamically unstable under isoionic conditions. When pH was shifted, either to acid or alkaline regions, with respect to the protein isoelectric pH, and ionic strength increased, two-phase systems became single-phase. System conditions, therefore, play an important role in the stability of protein-polysaccharide-water systems. Burkus (1996) observed thermodynamic incompatibility between barley β-glucan and whey proteins, but detailed investigation was not carried out. When formulating products, such as beverages, knowledge of the phase separation behavior of β-glucan and whey protein is essential to produce a stable, homogeneous product.

2.3.4. Health promoting properties of whey and whey proteins

Therapeutic applications for whey date back to ancient Greece, where in 460 B.C. Hippocrates prescribed whey for a variety of human illnesses. In the 1940's, whey was being applied in the treatment of dyspepsia, uremia, arthritis, gout, liver diseases, anemia and tuberculosis (Holsinger et al., 1974). Whey is not only used for treating illnesses, but concentrated whey proteins may also serve an important dietary role as an excellent source of protein.

The amino acid composition of whey protein contains all essential amino acids necessary in a healthy human diet. Whey protein has been described as the best dietary source of the branched chain amino acids, which have been described as important in the nutritional needs of athletes (Regester et al., 1996; Hoch, 1997). Whey protein was also described as having the highest bioavailability of protein sources, indicating that it is most effective in meeting the body's amino acid requirements, when compared with other proteins, including egg, beef, soy and casein (Werner, 1981). Therefore, whey protein-enriched foods and beverages may be beneficial for athletes and those involved in bodybuilding. Whey-enriched foods may be included in the diet, providing the protein required to aid the body in repairing damaged muscle tissue, as well as, facilitating its growth.

More recently, it is not the nutritional properties but the potential medicinal properties of whey proteins that are receiving the most attention (Regester et al., 1996; de Wit, 1998). Bovine serum albumins and immunoglobins may enhance the immunological effects of infant formula and also be used in the development of cow's milk infant formula closer in composition to that of human

breast milk (Bounous et al., 1988; de Wit, 1998). Lactoferrin and lactoperoxidase both show some antibacterial activity. Zydney (1998) referred to lactoferrin's ability to increase iron absorption in infant formula and potential anti-opoid and anti-thrombotic activity. Glycomacropeptide, a peptide fragment of casein, that is highly water soluble and therefore retained in the whey, has been referred to improve lipid digestion, protect against flu viruses, prevent the adhesion of tartar to teeth and inhibit the attachment of *Escherichia coli* to intestinal walls (Maubois and Olliver, 1997). WPC and WPI contain many growth factors, hormones, and enzymes (lipoprotein lipase, catalase, lysozyme, α-amylase, and superoxide dismutase). The biological activity of these components may lead to many applications as well (Zydney, 1998).

Anticarcinogenic effects of whey proteins were reported by Bounous et al. (1991). Rats, treated with dimethylhydrazine (DMH) to induce cancerous tumors, were shown to get less tumors when their diets were supplemented with whey proteins (McIntosh et al., 1995). More significantly, inhibition of breast cancer tumor growth and a reduction in the size of cancerous tumors were achieved when whey protein was added to cancer patient diets (Kennedy et al., 1995; Baruchel and Viau, 1996). There are also reports of whey applications for immunity enhancement and dietary treatment of AIDS related illnesses (Bounous et al., 1988). With a high protein bioavailability, there may be many applications for whey protein based foods that will help increase the absorbable dietary protein for those recovering or coping, with injuries or disease. Whey appears to be an excellent nutritional supplement, with many potential health-promoting

properties. Whey and whey protein enriched food products may, therefore, have a bright future.

2.4. FUNCTIONAL FOODS AND NUTRACEUTICALS

A major focus of the current food processing industry, and associated research and development, is the production of foods containing health-enhancing components that will improve consumer health beyond meeting basic nutritional requirements (Sloan, 1999). This trend arose both from research and development of the medicinal-like qualities of some food components and a population of consumers demanding such foods with hopes of improving their health. As has been quoted many times, especially more recently, "Let medicine be your food and food be your medicine", or simply "food-as-medicine" (Sloan, 1999), accurately describes the concept that is currently dominating consumers, research and industry.

Until recently, there was confusion regarding what exactly functional foods and nutraceuticals are. A legal definition of these terms is required so that consumers can be provided with food products with health claims that are supported by scientific research findings. Health Canada (1997), therefore, proposed to define a functional food as, "similar in appearance to conventional food, consumed as part of a usual diet, and demonstrates physiological benefits and/or reduces the risk of chronic disease beyond basic nutritional functions". This definition was developed to regulate what can be labeled and sold as a functional food in Canada. Current Canadian legislation, however, still does not

allow functional food products to be labeled with specific health claims.

The term "nutraceutical" arose to separate health promoting supplements and extracts from the claims associated with functional foods. Health Canada (1997) defines a nutraceutical as "a product produced from foods but sold in pills, powders, (potions) and other medicinal forms not generally associated with food and demonstrated to have a physiological benefit or provide protection against chronic disease." The increased use of herbal supplements, making herbal extracts of kava kava, St. John's Wort and *Echinacea* household names, is one of the factors which led to the need for the creation of this category of products (Swientek, 1998). Specific health claim labels are also not yet permitted for nutraceuticals sold in Canada.

It is necessary to differentiate between functional foods and nutraceuticals. As extracts of concentrated nutraceutical compounds from foods continue to be produced, the division between food and drug is becoming less definite. Regulation of these products will facilitate consumer safety and protect them from any "snake oil remedies" or false claims. Responsible research will help to educate the consumer and prevent any abuse or overdose of such extracts.

Despite the lack of labeling of specific health claims in Canada and limited labeling in the United States, the functional food and nutraceutical market is by far the fastest growing segment of the food industry. In the US market, sales of vitamin and herbal supplements have reached $12 billion/year, and energy drinks, bars, and powders, $3.14 billion/year (based on 1997-98 data) (Sloan, 1999). Traditionally fortified foods, functional foods, when generalized to include

fat-free and low-calorie food products, attain sales of $92 billion/year (Sloan, 1999). Many new products are being developed such as higher calcium yogurts, calcium/antioxidant juices, antioxidant-enriched eggs, energized teas and superoxygenated waters. More than half of consumers (52%) now believe food choices can aid in control of the use of drugs. This is up from 42% reported in 1994 (Sloan, 1999).

It can therefore, be expected that the development of new food products, with potential health-enhancing properties, should be welcome and successful in a demanding functional food and nutraceutical market. Consumer demands are changing faster than government legislation and encouraging further research and development of new products (Kuhn, 1998).

2.4.1. Functional foods

In the beginning of 1997, the Quaker Oats Company's (Quaker Oats) petition submitted to the Food and Drug Administration (FDA) of the United States was approved allowing Quaker Oats to label their oatmeal products with a claim emphasizing the health enhancing potential of oat fiber. This approval was based on the research findings in the area of the hypocholesterolemic effects of soluble fiber, found in the form of β-glucan in oats. Quaker Oats' new label states that "including oatmeal in a diet, low in cholesterol and saturated fats, may reduce the risk of developing coronary heart disease" (Klis, 1996). This move by Quaker Oats and the FDA set the stage for the labeling of other such products with specific health claims.

Psyllium husk was the second product to receive FDA permission for

labeling with health specific claims. The soluble fibre in psyllium husk, like oat soluble fibre, has been shown to lower serum cholesterol when included in the diet (Davidson et al., 1998; MacMahon and Carless, 1998). Psyllium is the active ingredient in Kellogg's *Ensemble* line and Bran Buds breakfast cereal, which will also have a "heart healthy", functional food, label similar to that of Quaker Oats (Kuhn, 1998; Sloan 1999). Soy, also with a cholesterol-lowering claim before the FDA, is the next functional food product expected to benefit from FDA approved health-enhancing labeling (Sloan, 1999).

2.4.2. Functional beverages

The variety of beverages being produced is rapidly changing. The market shelves and coolers no longer have only soda pop, juices and dairy beverages. The products available are now much more diverse providing the consumer a choice of a variety of ready-to-drink meal replacement drinks, sports drinks, iced teas and coffees, herbal teas, frozen carbonated beverages, vegetable juices, fruit blends, smoothies, and the list continues on. Soft drinks have traditionally ranked number one in sales. However, in recent years consumers have been opting for more exotic beverages such as the iced coffees, teas, isotonic or sports drinks and non-carbonated fruit beverages (Giese, 1992).

Beverages normally do not compose a whole meal, but rather complement a meal. They are, therefore, an excellent medium for the introduction and enrichment of our diets with nutraceutical compounds, without making drastic changes to our daily meals. By adding a nutraceutical ingredient to a beverage, traditional beverages are transformed into functional beverages, possessing the

health enhancing abilities. For classification and potential labeling as a functional beverage, the product must obey the functional food definition set out by Health Canada (1997), outlined earlier (section 2.4).There are excellent opportunities in the beverage market for functional beverages. Snapple, for example, reported a 113% increase in herbal enhanced beverage sales for the May 1997 to May 1998 period (Swientek, 1998). Antioxidant- and calcium-fortified juice sales have grown at double the rate of traditional juices (Sloan, 1999). Extracts of ginkgo biloba, ginseng, echinacea, guarana, and other herbs and botanicals are being added to beverages, such as sports drinks, soft drinks, teas and juices, in hopes of capturing the consumer's attention by implying health or performance enhancing potential (Kuhn, 1998; Pszczola, 1998).

2.4.2.1. Beverage ingredients

The production of a beverage requires many ingredients and careful combination of these ingredients. The goal of the beverage producer is to provide a beverage with a refreshing taste, a stable and safe product with consumer appeal while minimizing costs. Ingredients are, therefore, carefully chosen. A list of typical ingredients, that may be found in a fruit beverage and their functions are displayed in Table 2.5.

All beverages contain water whether originating from whole fruit or added (Hicks, 1990). Water represents the bulk or mass of the beverage, typically representing 85 to 93% of the total volume, and up to 98% in diet drinks (Giese, 1992). The end quality of the beverage is dependent upon the quality of the water

Table 2.5. Principal ingredients of a typical fruit beverage.[1]

Ingredients	Function
Water	Bulk and mass Solvent/carrier Thirst quenching
Sugars	Flavor sweetness fruitiness Mouthfeel/body Nutrition Facilitate water absorption (Appearance and preservation in syrups)
Fruit	Flavor Appearance (Nutrition)
Nutrient additions including salts	Nutrition; ascorbic acid and tocopherols are also antioxidants Contolled absorption of sugars and water
Acids	Flavor Antimicrobial effect
Flavorings Artificial sweeteners Colorings Emulsifiers and stabilizers	Flavor Appearance; carotene and riboflavin colorings are also nutrients
Antioxidants	Improved flavor and vitamin stability
Preservatives	Antimicrobial effect Sulphite also has anti-browning and antioxidant effectiveness
Acidity Regulators	Improved dental safety Reduced can corrosion

[1] Adapted from Hicks (1990).

used. Clean, potable water is required for a good product. Manufacturers may take the domestic water and use filtration and other processes to ensure that only the cleanest water is used to formulate their products (Woodroof and Phillips, 1981b). This way no contaminants, off-flavors or spoilage organisms originating in poor quality water will be present to ruin the final product.

A beverage formulation usually requires the addition of sweeteners, except in cases where the naturally occurring sweetness of fruit juices, if used, is sufficient. Sweeteners provide the characteristic sweet taste to juices and enhance body or mouthfeel (Hicks, 1991; Giese, 1992). There are many types of sweeteners that may be used depending on the application. Typical beverage sweeteners include sucrose, glucose, fructose, high fructose corn syrup and invert sugar. However, if a low-calorie product is to be formulated then a high potency, artificial sweetener such as aspartame, acesulfame-K, cyclamate or saccharin may be used (Woodruff and Phillips, 1981a; Hicks, 1990; Giese, 1992). These sweeteners add no calories, have a sweet taste and are suitable for reduced calorie or diabetic diets.

Natural and/or artificial flavor is what gives each drink its character and attracts consumers to try them. There are many flavors to choose from. These flavors may be natural extracts, nature identical or artificial flavorings (Hicks, 1990; Giese, 1992). Single fruit flavorings and now the popular fruit blends and, fruit and vegetable blends are available. Traditional juice flavors such as apple and orange still rank highest as flavors of choice. However, other more exotic flavors, such as mango, are rising in popularity (Giese, 1995; Swientek, 1998).

This change is reflected in the flavors available and beverages that are currently being produced.

Colorants, like flavors, can be either natural extracts, nature identical or artificial (Giese, 1992). β-Carotene and anthocyanins are colorants of natural origin and demonstrate nutraceutical potential based on antioxidant activity (Giese, 1995). There are, however, many acceptable artificial colorants that are used, and available at much lower cost. A clear glass or plastic bottle allows the consumer to see the beverage color, increasing consumer interest.

Acids give a tart, refreshing character to beverages. They also provide antimicrobial protection and reduce or eliminate the need for added preservatives, such as sodium benzoate (Giese, 1992). Beverage acidulants include citric, ascorbic and malic acids. Each acid imparts its own flavor characteristics and sourness to the final product (Hartwig and McDaniel, 1995). However, citric acid, occurring naturally in fruits, is the preferred acidulant for fruit-flavored beverages (Giese, 1992).

Other beverage ingredients may include thickeners for altering mouthfeel, especially for use with high potency sweeteners (Giese, 1995). Traditional sweeteners make 10 – 20% of the final product and contribute both viscosity and mouthfeel. When high potency sweeteners are used less of the sweetener is needed, 10-1000 ppm. Therefore, the beverage loses viscosity and mouthfeel (Hicks, 1990). The addition of thickeners can correct this and provide a better mouthfeel for the beverage. Thickening agents may also function as stabilizers in beverage formulations. Some beverages, especially those involving mixes of dairy

and fruit juices, require a stabilizer to produce a homogenous product without phase separation (Mann, 1996). The thickening and stabilization properties of barley β-glucan may be advantageous in a beverage formulation, however, there is no data available on its behavior in such an application.

Recently, herbal extracts are being used as ingredients in beverages. Extracts of the herbs *Ginkgo biloba*, guarana, kava kava, *Echinacea*, St. John's wort, and others, are added to boost the potential for labeling with specific health claims for functional beverages and attract consumer attention (Swientek, 1998, Pszczola, 1998). Ready-to-drink iced herbal teas are gaining in popularity, and fruit-flavored beverages, serve as an excellent medium for the inclusion of nutraceutical extracts. Barley β-glucan, an ingredient displaying nutraceutical potential, could also be used in functional beverages.

2.4.2.2. Beverage production

The manufacturing of beverages requires a large plant with specialized pieces of equipment arranged for continuous production. Incoming ingredients and packaging materials must be received and stored appropriately whether refrigerated or not. In modern plants, all beverage ingredients are combined and flow from receiving to packaging in a continuous manner.

Beverage ingredients must first be combined together, according to the formulation set by the product developers. This is accomplished in large tanks where ingredients are added to water and mixed at shear rates adequate to ensure complete homogeneity of the beverage mixture. Large steam jacketed kettles, with mixers, may be used to control temperature and mix ingredients. The

contents of the tanks may then be pumped into in-line homogenizers, if necessary. A homogenizer is required to disperse and stabilize any lipid soluble ingredients, such as flavor extracts, by dispersing the extract into small droplets. According to Stokes' law (eq. 2.1), as the size of the dispersed droplets decreases, the stability of the emulsion (i.e. lipid-soluble extract in water) increases. Homogenization will also aid in the stabilization of texture ingredients and clouding agents. This will ensure a stable beverage that remains a single phase (Solberg et al., 1991).

Pasteurization is used to achieve a microbiologically safe and stable beverage. Pasteurization is the heat treatment of a product for an appropriate time at a temperature high enough to significantly reduce the number of pathogenic organisms that may be present in the product (Castaigne and Goulet, 1985). The beverage pH and use of a preservative influence the temperature/time treatment required to produce the desired effect. For example, a product with a preservative, such as sodium benzoate, and/or a low pH will have inherent protection from microbial spoilage and, therefore, not require a severe heat treatment. These conditions will stop or delay the growth of any spores or microbes that may have survived pasteurization. Juice and beverage pasteurization is usually done at 92-95°C with a 20-30 second holding time at that temperature (Solberg et al., 1991).

Pasteurization may be done in one of three ways, as outlined by Hicks (1990). In-line flash pasteurization, or aseptic processing, may be used as the product is pumped from the mixing tanks to the homogenizer, and if necessary, to packaging where it is bottled or canned. This process involves rapid heating of the product in a heat exchanger, which transfers heat from out-flowing hot liquid (i.e.

pasteurized) to incoming cool liquid (i.e. unpasteurized), and holding the product for a set time at a set temperature. Rapid heating and cooling help minimize any heat-induced changes in beverage quality. However, with this method the packaging material must be sterilized to ensure no contamination of the incoming pasteurized product. In-pack pasteurization may also be used. In this method, the package is filled and closed. The whole package and product are then heated and quickly cooled. The third type of pasteurization is known as hot filling, where the package is filled with product at the desired temperature, closed and then inverted to ensure that all areas inside the package come in contact with the hot product.

Packaged products will then proceed to have a label attached. The container and final packaging are the final and important component in the production of a beverage product. The container and label design give the product identity and aid the consumer in making purchasing decisions. The label will soon play an important role in informing the consumer of the specific health promoting claims of each product. However, until Canadian legislation allows such labeling, the functional food and nutraceutical market relies on consumers who know the activity of ingredients listed on the product label.

The bottles or cans are then boxed and palletized, requiring either workers or specialized packaging and palletizing machines to perform these tasks. The pallets of finished product are then moved to storage until shipped to supply warehouses and retail outlets (Phillips and Woodroof, 1981; Hicks, 1990).

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3. FUNCTIONAL BEHAVIOR OF BARLEY β-GLUCAN: VISCOSITY AND THE EFFECTS OF pH/TEMPERATURE, SUGAR, AND WHEY PROTEIN ISOLATE[1]

3.1. INTRODUCTION

Mixed linkage (1→3), (1→4)-β-D-glucan (β-glucan), is a polysaccharide and soluble fiber component found predominantly in the cell walls of cereal grains, most notably barley and oats. Although research is limited, β-glucan gum has been shown to possess many potentially useful functional properties including, thickening, stabilization, emulsification and gelation (Dawkins and Nnanna, 1995; Burkus, 1996; Burkus and Temelli, 1999a and 1999b). These functional properties are the result of the β-glucan's water solubility and ability to form viscous hydrocolloid sols.

It is the viscosity or thickening effect of β-glucan, which aids in the stabilization of emulsions or particles within a solution. According to Stokes' law (eq. 2.1), as viscosity increases, the precipitation of particles in a solution or re-aggregation of the dispersed phase within the continuous phase of an emulsion, are slowed down or stopped in the case of a completely stable system (Nawar, 1985). Gelation of β-glucan will occur when sufficient association among long polymer chains of β-glucan forms a three-dimensional network through hydrogen bonding (Glicksman, 1982; Burkus, 1996; Burkus and Temelli, 1999a). Water becomes trapped in the network structure and the gel structure is formed.

[1]A version of this chapter is to be submitted to Food Hydrocolloids for consideration for publication.

The viscosity and rheological behavior of water-soluble polysaccharides, such as barley β-glucan, are affected by many variables, including temperature. As temperature increases, the molecular motion increases within the sol due to higher energy levels. Interactions between polysaccharide chains are, therefore, decreased, and viscosity is reduced. At lower temperatures, polymer interaction is more likely to occur with the decrease in molecular motion, creating more internal friction leading to an increase in viscosity (Glicksman, 1982; Whistler and Daniel, 1985).

Rheological behavior of sols is also affected with changes in temperature. A sol, prepared with a polysaccharide such as β-glucan, may demonstrate pseudo-plasticity, or shear thinning with increasing shear rate, at lower temperatures. However, it may become Newtonian (or constant viscosity) at higher temperatures (Glicksman, 1982; Dickinson and Stainsby, 1982; Dawkins and Nnanna, 1995).

The molecular weight (MW) of the polymer will also affect viscosity. MW is positively associated with viscosity, therefore, as MW increases viscosity increases (Glicksman, 1982). Changes in the functional properties of a polysaccharide, such as β-glucan, may occur with extraction and processing treatments, especially with changes in pH and temperature (Burkus and Temelli, 1998). Polysaccharides typically will undergo acid-cut hydrolysis at acidic pH and elevated temperatures (Whistler and Daniel, 1985). As a result of hydrolysis, MW of the polysaccharide chains decreases as well as sol viscosity. Such changes in functional behavior affect potential food and beverage applications.

The viscosity and functionality of sols can also vary depending on the presence of, and interaction with, other sol components. For example, the addition of sucrose to water, as in beverage production, results in an increase in viscosity, giving the product body or mouthfeel (Hicks, 1990). When sugar is added to a polysaccharide sol, such as a β-glucan sol, viscosity will be further increased (Dawkins and Nnanna, 1995). A viscosity increase, greater than the sum of the individual viscosity effects of sucrose and polysaccharide alone, may be observed (Pangborn et al., 1972; Glicksman, 1982). Such a synergistic effect may be due to interactions between the sucrose and polysaccharide.

If sucrose is added at high enough concentrations, however, a decrease in sol viscosity will be observed. Viscosity of oat β-glucan sols decreased, when sucrose was added at concentrations >65% (Dawkins and Nnanna, 1995). This viscosity decrease is due to the competition for water that occurs between the polysaccharide and sucrose, leading to decreased polymer hydration (Vaisey et al., 1969; Dawkins and Nnanna, 1995). The polysaccharide, therefore, becomes less extended in solution, resulting in a reduction in internal friction and, therefore, viscosity. Interactions, affecting functional properties of β-glucan, may also occur with food components such as other polysaccharides, salts or proteins (Whistler and Daniel, 1985; Burkus, 1996).

The combination of proteins and polysaccharides in solution results in one of three scenarios, co-solubility, thermodynamic compatibility (formation of soluble or insoluble complexes) or thermodynamic incompatibility (section 2.3.3). Co-solubility is the least typical scenario. The formation of insoluble complexes

or incompatibility of biopolymers results in sol instability and phase separation. Incompatibility may be due to many variables including, the characteristics of the protein and polysaccharide chosen, the concentrations of biopolymers, pH, and ionic strength (Tolstoguzov, 1991). Grinberg and Tolstoguzov (1972) studied the interactions of gelatin with D-glucans (amylopectin, glycogen and dextran), similar in composition to β-glucan.

Currently, there is limited research on β-glucan functional properties and β-glucan-ingredient interactions. Burkus and Temelli (1999a, 1999b) have demonstrated gelation of barley β-glucan and its potential in the stabilization of foams and emulsions. Dawkins and Nnanna (1995) have also determined the effects of sucrose on β-glucan rich oat gum. Preliminary results of Burkus (1996) demonstrate the incompatibility that exists between barley β-glucan and other components such as whey protein concentrate and starch. However, the literature lacks detailed information on the interactions of barley β-glucan with sugar or whey proteins, which are important for potential applications in food and beverage formulations. In addition, our understanding of the effects of pH/temperature processing treatments on the viscosity of β-glucan sols is limited. Such information is required to characterize barley β-glucan, determine its functionality to assess potential food and beverage applications and develop β-glucan enriched products. The objectives of this study were, therefore:

1. to determine the effects of sucrose and whey protein isolate (WPI) on the viscosity of barley β-glucan sols,
2. to examine the possible interactions between WPI and β-glucan, and

3. to determine the effect of pH/temperature treatments on the viscosity of barley β-glucan sols in an effort to assess processing stability.

3.2. MATERIALS AND METHODS

3.2.1. Materials

Barley β-glucan concentrate (β-glucan gum) was obtained from waxy, hulless barley (Bly Blend, mix of two experimental barley varieties, SB89528 and SB89497), at the POS Pilot Plant Corp. (Saskatoon, SK), according to Burkus and Temelli (1998), prior to this study. Sucrose was obtained from a local retail grocery store. WPI, Alacen 895, was provided by New Zealand Milk Products (Santa Rosa, CA).

3.2.2. Compositional analyses

Carbohydrate components of the β-glucan gum, including starch, insoluble fibre, and β-glucan, were determined according to AACC method 32-07 (AACC, 1984), McCleary et al. (1994) and McCleary and Glennie-Holmes (1985), respectively, using Megazyme assay kits (Megazyme, Bray, Ireland). Ash and moisture contents were determined using AACC method 08-01 (AACC, 1984) and according to McCleary and Glennie-Holmes (1985), respectively. Protein and lipid contents were determined using Kjeldahl and Goldfisch apparatus, AACC method 46-11 and AACC method 30-25, respectively (AACC, 1984). Total pentosans were determined according to Hashimoto et al. (1987). All analyses were performed in triplicate and results were reported on dry matter basis.

3.2.3. Preparation of sols

β-Glucan sols were prepared as follows: β-Glucan gum was added slowly to pre-heated water, stirring on a magnetic stir plate. The sols, covered with aluminum foil, were brought up to boiling temperature and boiled for approximately 1 minute, ensuring no boiling-over. Sols were then cooled to room temperature with continued stirring. The final weight of sols was then adjusted to pre-boiling weight, by adding water to account for moisture losses during boiling and cooling steps. All concentrations are expressed in weight percentage (% w/w).

Sucrose was added to β-glucan sols only after the hydration procedure was completed to ensure full hydration and extension of the β-glucan polymers. Sols containing 0.5 and 1.0% β-glucan gum, with and without 20% sucrose, were prepared at pH 4.5 for viscosity determination. WPI was also added to β-glucan sols after the hydration procedure and upon cooling to room temperature to avoid denaturation of the heat sensitive whey proteins. Sols containing all combinations of 0.25, 0.50, 0.75 or 1.00% β-glucan gum and 0.5, 1.0, 3.0 or 5.0% WPI were prepared at pH 3.0 for viscosity measurement and examination of WPI/β-glucan interaction.

Sol pH was adjusted using 2N HCl and 2N NaOH and was measured using a Corning pH meter (model 220, Corning Labware and Equipment, Corning, NY). The pH meter was calibrated using a two-point calibration, at pH 2.0 and pH 7.0.

3.2.4. Rheology of β-glucan sols

Rheological measurements of β-glucan sols with and without sucrose were determined using a Haake Rotoviscometer (model RV-3, Gebruder Haake, Berlin, Germany) at 5 and 25°C. Complete flow curve measurements with increasing shear rate, were recorded to determine flow behavior and viscosity (calculated as the slope of Newtonian flow curves). The viscosity of β-glucan/WPI sols were measured at constant shear rate, at 5°C. Two replicate sols were prepared for the determination of viscosity. Duplicate measures were recorded for each sample.

3.2.5. β-Glucan-whey protein interactions

Absorbance, measured at 900 nm using a diode array spectrophotometer (model HP 8452A, Hewlett Packard, Boise, ID), was used to examine the thermal stability of WPI. Absorbance of whey protein solutions increases with denaturation and aggregation (Patocka et al., 1986). WPI solutions, 0.5, 1.0 and 5.0%, were prepared, pH was adjusted to 3.0 and 7.0, and heat treatment (83°C/10 min) was applied. Absorbance was then recorded. Solutions not undergoing heat treatment served as control.

Differential scanning calorimetry (DSC), using a Du Pont 910 Differential Scanning Calorimeter, set at 5°C/min, with Du Pont 990 Thermal Analyzer (Du Pont Instruments, Wilmington, DE), was used to examine the thermal properties and interactions of β-glucan gum/WPI sols. Sols were prepared at pH 3.0 and 7.0 at concentrations that allowed DSC samples (10% WPI solution with 0 or 0.5% β-glucan gum), approximately 20-25 mg, to contain 2-3 mg of dry matter.

β-Glucan gum/WPI sols, for examination of thermodynamic

compatibility, were prepared as described earlier. Sols were then transferred into separatory funnels and stored at 5°C for two days. Phases of sols that demonstrated instability, were separated using the separatory funnels. Regions around the phase boundary where phases began to mix during separation, were discarded. Protein and β-glucan contents of each phase were determined.

3.2.6. pH/temperature treatments

The sols used to determine the effect of pH/temperature treatments on viscosity were prepared as follows: 0.5% β-glucan sols were adjusted to pH 3, 5, 7 or 9 and transferred into polypropylene test tubes. The tubes were lightly capped and held in a gently shaking water bath at 55, 75 or 95°C for 30 min. The two extremes, pH 3 and 9 treatments, held at elevated temperatures for 30 min, were selected to examine the effects of processing of high acid foods and beverages (e.g. pasteurization and hot-fill processing) containing β-glucan and alkali extraction conditions on β-glucan, respectively. The temperature of each sol was monitored and recorded using thermocouples attached to a data recorder. At the end of 30 min, sols were transferred to a cold water bath and cooled to room temperature. The experiment was replicated twice. Viscosity measurements were then taken in duplicate. Sols at all four pH levels, without heat treatment, served as controls.

3.2.7. Statistical analysis

Analysis of variance of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 7 (SAS Institute, 1989). Multiple comparison of the means was performed by Student-Newman-Keuls

(SNK) test, at α=0.05 level.

3.3. RESULTS AND DISCUSSION

3.3.1. Composition of barley β-glucan gum

The composition of the β-glucan gum extracted at the POS Pilot Plant Corp. is presented in Table 3.1. β-Glucan content of the gum was 85.63% (w/w). A small amount of precipitate was present in β-glucan sols. The residual proteins or insoluble fiber components, composing 1.41% and 1.86% of the gum, respectively, may be the source of the precipitate in the sols.

3.3.2. Rheology of β-glucan sols

The characteristic rheological behavior observed for 0.5 and 1.0% β-glucan sols at 5 and 25°C is displayed in Figure 3.1. Sols exhibited Newtonian behavior based on a linear relationship on shear stress vs. shear rate plot (R^2=0.99), at both concentrations and temperatures. The sols, therefore, maintained constant viscosity within the shear rate ranges studied.

At 5°C, the viscosity of 0.5 and 1.0% sols was 8.25 and 26.14 mPa·s, respectively. When the temperature was raised to 25°C, viscosity dropped to 4.41 mPa·s for 0.5% and 11.56 mPa·s for 1.0% sols. By increasing temperature, polymer-polymer interaction and viscosity are decreased (Whistler and Daniel, 1985). This may be causing the disassembly of the fringed micelle structures of β-glucan described by Linemann and Kruger (1998). Consequently, the effective MW of the network of polysaccharide units is reduced down to the MW of individual β-glucan polymers, leading to a reduction in viscosity.

Table 3.1. Composition of barley β-glucan gum.

Component	Concentration[1] % (w/w)
Moisture	4.79
Carbohydrates	
β-Glucan	85.63
Pentosans	6.22
Starch	1.14
Insoluble Fibre	1.86
Protein	1.41
Lipid	0.007
Ash	3.90

[1]All concentrations except moisture expressed on dry matter basis.

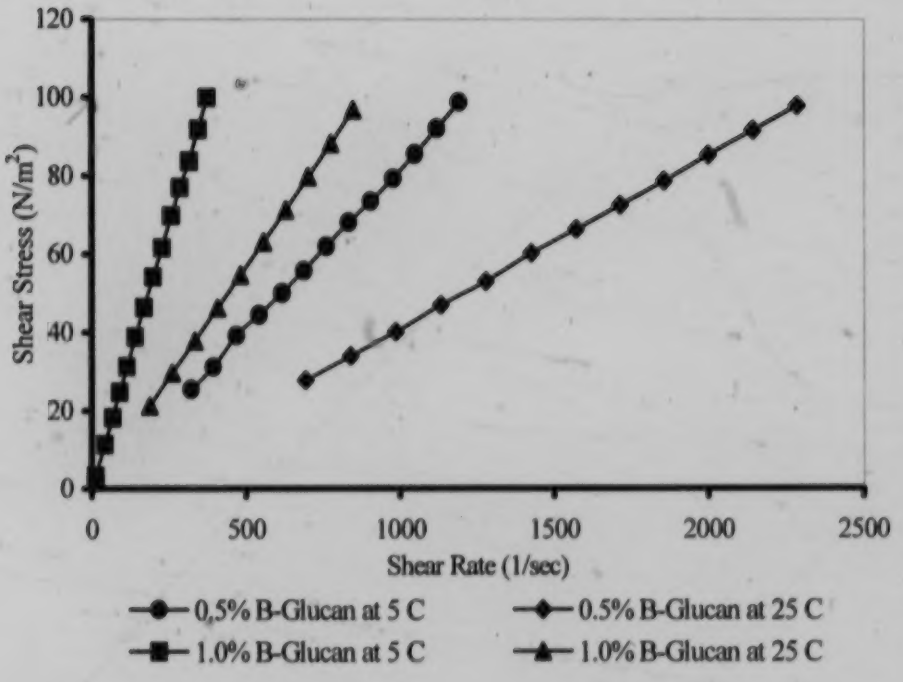

Figure 3.1. Effect of temperature on flow behavior of 0.5 and 1.0% (w/w) β-glucan sols at pH 4.5.

Effect of sucrose

The typical rheological behavior observed for β-glucan sols with 20% sucrose added is depicted in Figure 3.2. These sols, displaying linear shear stress vs. shear rate relationship (R^2=0.99), exhibit Newtonian behavior for the shear rate ranges studied, similar to β-glucan sols without sucrose (Fig. 3.1). Sucrose addition, therefore, did not result in changes in the rheological behavior of β-glucan sols. A comparison of the viscosity of β-glucan sols (0.5 and 1.0%) at 5 and 25°C, with and without 20% sucrose addition is given in Table 3.2. The viscosity of β-glucan sols was significantly increased ($p \leq 0.05$) with the addition of 20% (w/w) sucrose. These results are in agreement with those reported for oat gum, also rich in β-glucan (Dawkins and Nnanna, 1995), where sucrose addition at 0-65% was found to increase viscosity. The addition of sucrose to barley β-glucan sols resulted in a larger than expected increase in sol viscosity. Synergism between hydrocolloid and sugar occurs when interactions produce a viscosity increase greater than the sum viscosity of component hydrocolloid sol and sugar solution (Glicksman, 1982). Interactions may have occurred among barley β-glucan and sucrose, producing the observed increase in viscosity greater than the summed viscosity of 20% sucrose and 0.5 or 1.0% β-glucan sol (Table 3.2). This increase is especially apparent at the low temperature and high β-glucan concentration. However, it is not clear if this is due to a synergistic effect.

Effect of whey protein isolate

The effect of WPI addition on β-glucan sol viscosity determined at 5°C, pH 3 and 64 rpm (shear rate of 340.83 - 344.08 sec^{-1}) is shown in Figure 3.3. The

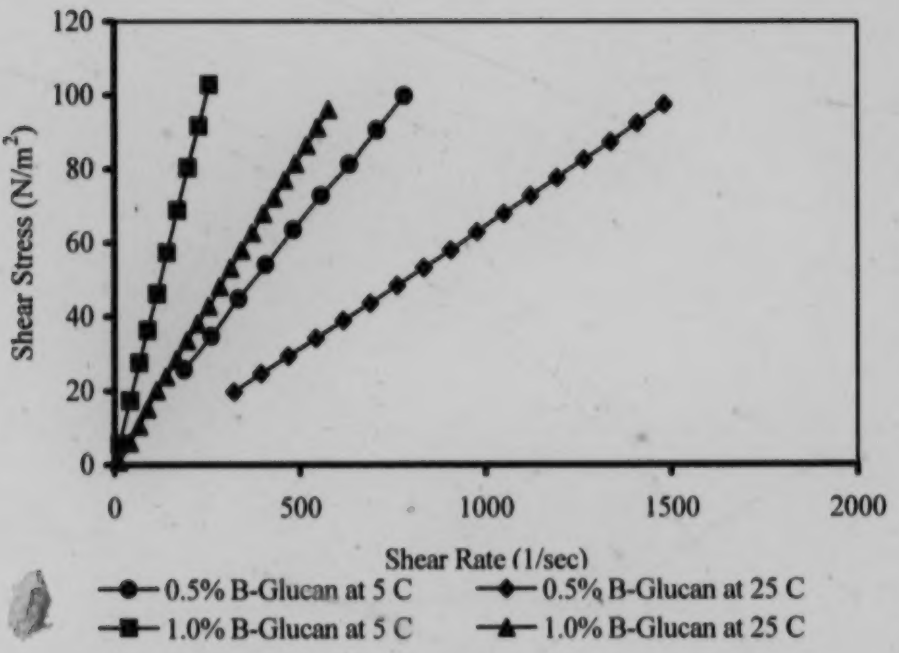

Figure 3.2. Effect of temperature on flow behavior of 0.5 and 1.0% (w/w) β-glucan sols with 20% (w/w) sucrose at pH 4.5.

Table 3.2. Comparison of the viscosity of 0.5% and 1.0% (w/w) β-glucan sols with and without 20% (w/w) sucrose addition at 5 and 25°C.

β-Glucan Gum Concentration (%)	Sucrose Concentration (%)	Temperature (°C)	Viscosity[1,2] (mPa·s)
0	20	5	3.24 ± 0.06
0	20	25	1.76 ± 0.03
0.5	0	5	8.26 ± 0.15
0.5	0	25	4.41 ± 0.07
0.5	20	5	12.93 ± 0.46
0.5	20	25	6.61 ± 0.09
1.0	0	5	26.14 ± 0.38
1.0	0	25	11.56 ± 0.30
1.0	20	5	40.58 ± 0.70
1.0	20	25	16.87 ± 0.42

[1]Viscosity of 20% (w/w) sucrose measured at 256 rpm (constant shear rate).
[2]Viscosity of β-glucan gum and β-glucan gum/sucrose sols determined from the slope of the shear stress-shear rate flow curves. Mean ± standard deviation reported based on 4 determinations.

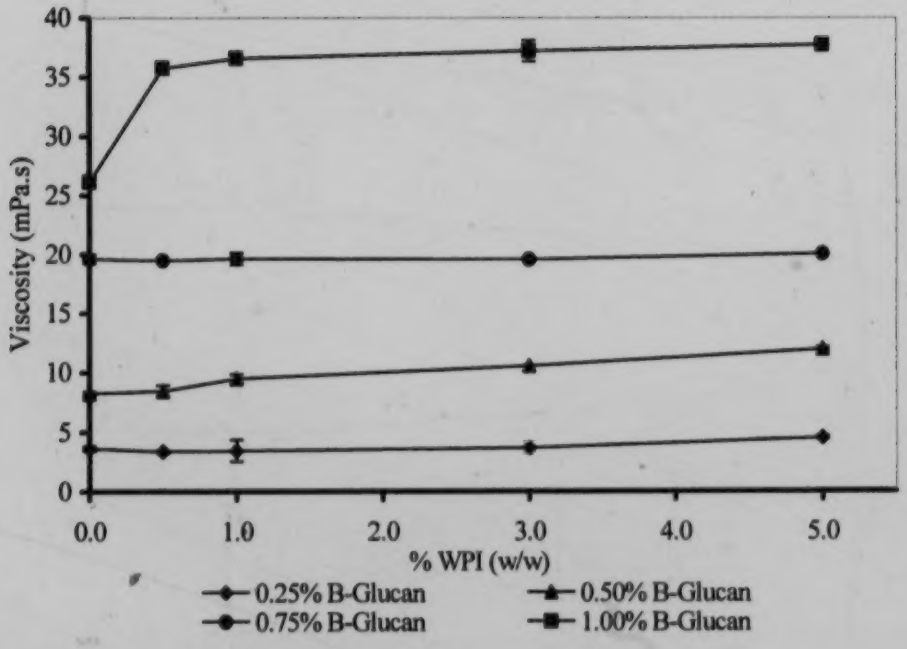

Figure 3.3. Effect of WPI addition on β-glucan sol viscosity measured at 5°C and 64 rpm. Means of 4 determinations are reported.

addition of 0.25, 0.50, 0.75 and 1.0% WPI, which alone yielded sol viscosity of 2.22, 2.23, 2.63 and 2.63 mPa·S, respectively, to 0.5 and 1.0% β-glucan sols significantly increased ($p \leq 0.05$) viscosity. A small increase was observed with WPI addition to the 0.5% β-glucan sol. However, the relatively large increase in viscosity observed with the addition of WPI to the 1.0% β-glucan sol implies some interactions may have occurred at this level of β-glucan and produced the large viscosity increase. The viscosity of the 0.25% gum sol was significantly increased ($p \leq 0.05$) only with 5.0% WPI addition. WPI addition increased the viscosity of 0.75% β-glucan sol only slightly.

The large increase in viscosity observed when WPI was added to 1.0% β-glucan sols indicates that there may be interactions between β-glucan polymers and whey proteins. These results, however, suggest this only occurs when a threshold concentration is exceeded, since lower levels of β-glucan gum did not produce large increases in viscosity. Interactions may be of the same nature as the polymer-polymer interactions, hydrogen bonding and formation of the fringed micelle superstructures described by Linemann and Kruger (1998). Hydrogen bonding may also exist between β-glucan polymers and whey proteins. This results in trapping of both water and whey proteins in the β-glucan polymer network. The observable effect is an increase in sol viscosity.

3.3.3. β-Glucan-whey protein interactions

Solutions of WPI at pH 3 were more heat stable than WPI solutions at pH 7. This is indicated by the lower absorbance, less denaturation of whey proteins, for WPI solutions at pH 3 vs. pH 7, after heat treatment (Table 3.3). This agrees

Table 3.3. Absorbance[1] of whey protein isolate sols as a measure of protein aggregation.

WPI Concentration (%)	pH	Absorbance at 900 nm	
		Without heat	With heat[2]
0.5	3	0.035	0.024
1.0	3	0.066	0.043
5.0	3	0.368	0.291
0.5	7	0.031	0.049
1.0	7	0.070	0.091
5.0	7	0.268	0.344

[1] Means of 4 determinations are reported.
[2] Sols were held for 10 minutes at 83°C.

with the findings of Patocka et al. (1986) which showed that raw acid whey (pH 4.6-4.8) was more heat stable than sweet whey (pH 5.9-6.4) (Table 2.3). The decrease in absorbance observed for pH 3 solutions may be due to increased WPI solubility after heat treatment.

Careful examination of the DSC results (typical DSC curves obtained at pH 3 and 7 are shown in Figs. 3.4 and 3.5, respectively), for β-glucan/WPI sols, demonstrates some enhancement of heat stability. The addition of 0.5% β-glucan gum resulted in 2 and 3°C increase in the denaturation temperature of whey protein at pH 3 and 7, respectively. At pH 3 whey proteins denatured at 89 and 87°C, whereas at pH 7, denaturation temperature was 80 and 77°C, with and without β-glucan gum addition, respectively. The size of the endothermic peak for whey protein denaturation increased significantly ($p \leq 0.05$) for WPI at pH 3 and 7 with the addition of β-glucan gum. An increase in enthalpy, from 12.99 kJ/kg to 15.17 kJ/kg was observed with β-glucan gum addition to WPI at pH 3, while the enthalpy increase was from 3.62 kJ/kg to 4.95 kJ/kg at pH 7. The increase in the size of the endothermic peak for whey protein denaturation indicates that more energy input is required for denaturation to occur. This finding, along with the slight increase observed in denaturation temperature, indicate that β-glucan gum enhances the heat stability of whey proteins.

Increasing the ratio of gum to protein may further enhance protein stability. This increase in stability, expressed as an increase in denaturation temperature of whey protein and increase in energy required for denaturation, may be due to the formation of a more tightly arranged β-glucan polymer network

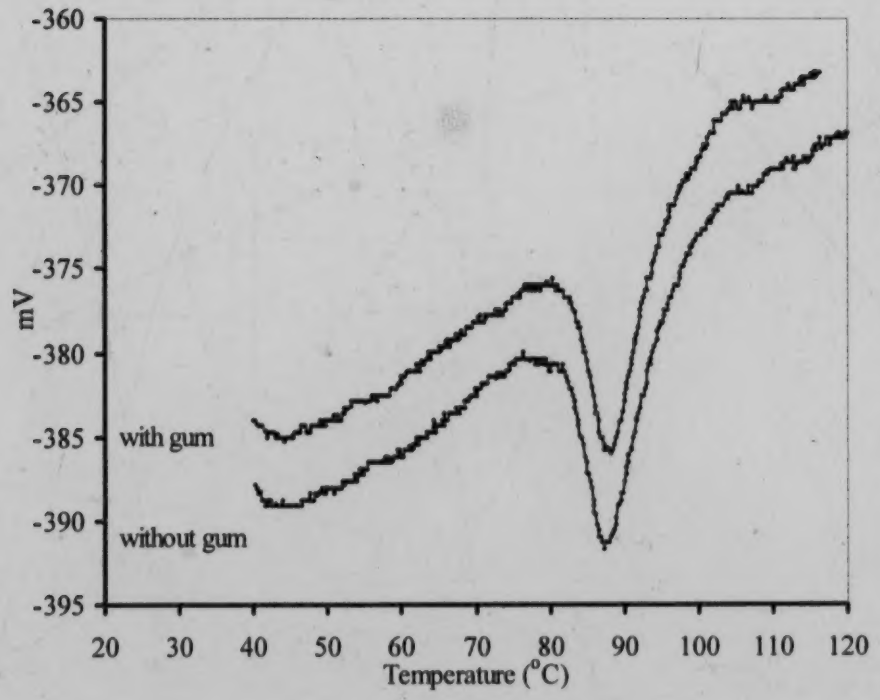

Figure 3.4. DSC results for 10% (w/w) WPI with and without 0.5% (w/w) β-glucan gum at pH 3.

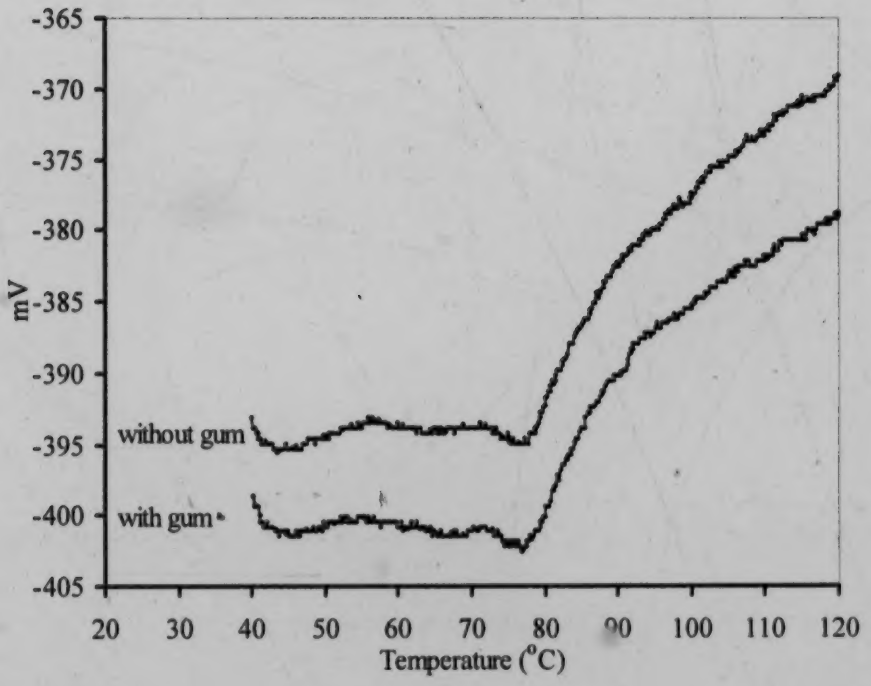

Figure 3.5. DSC results for 10% (w/w) WPI with and without 0.5% (w/w) β-glucan gum at pH 7.

produced with higher concentrations of β-glucan gum. The presence of an increased β-glucan polymer network could, therefore, act as a physical barrier inhibiting the aggregation of whey proteins.

Phase separation

The composition of initial β-glucan/WPI sols, as well as, the phases that separated after 2 days of refrigerated storage, is presented in Table 3.4. Compositional analysis of the phases revealed that the β-glucan appears to be more uniformly distributed throughout the two phases whereas protein is somewhat more concentrated in the lower phase. Some WPI/β-glucan gum sols, however, remained as stable single-phase sols, especially at the lowest level of β-glucan gum (0.25%). This concentration of β-glucan was, therefore, below phase separation threshold, regardless of WPI addition, and no separation occurred.

The addition of 0.5% WPI to a 0.5% β-glucan gum sol resulted in separation. However, when 1.0% WPI was added to a 0.5% β-glucan gum sol, no separation occurred. This is contrary to what is expected. The addition of higher concentrations of incompatible ingredients should result in more rapid separation. The significantly ($p \leq 0.05$) larger increase in viscosity that occurs with 1.0% WPI addition to a 0.5% β-glucan sol, compared to 0.5% β-glucan with 0.5% WPI (Fig. 3.3), may have temporarily stabilized the system. This is in agreement with Stokes' law (eq. 2.1), where increased viscosity slows separation velocity. However, when 1.0% WPI was added to 0.75 and 1.0% β-glucan gum sols, separation occurred. In these sols, the repulsive forces between phases were greater than the stabilization effect of viscosity increase.

Table 3.4. β-Glucan and whey protein concentration (w/w) of single phase and phase separated sols.

% WPI	% β-Glucan Gum	Phase[1]	%β-Glucan[2]	%Protein[2]
0.5	0.25	1	0.16 ± 0.00	0.52 ± 0.01
0.5	0.50	2a	0.32 ± 0.04	0.47 ± 0.03
		2b	0.32 ± 0.12	1.07 ± 0.59
0.5	0.75	3a	0.45 ± 0.06	0.33 ± 0.02
		3b	0.49 ± 0.04	1.54 ± 0.20
0.5	1.00	4a	0.65 ± 0.06	0.30 ± 0.03
		4b	0.65 ± 0.05	0.97 ± 0.45
1.0	0.25	5	0.16 ± 0.00	1.23 ± 0.06
1.0	0.50	6	0.32 ± 0.01	1.30 ± 0.47
1.0	0.75	7a	0.50 ± 0.01	0.95 ± 0.13
		7b	0.53 ± 0.01	1.51 ± 0.79
1.0	1.00	8a	0.65 ± 0.06	0.91 ± 0.20
		8b	0.58 ± 0.08	1.01 ± 0.06
3.0	0.25	9	0.13 ± 0.01	3.92 ± 0.81
3.0	0.50	10a	0.29 ± 0.06	3.30 ± 0.81
		10b	0.28 ± 0.04	3.33 ± 0.19
3.0	0.75	11a	0.51 ± 0.01	2.60 ± 0.35
		11b	0.49 ± 0.02	3.20 ± 0.01
3.0	1.00	12a	0.52 ± 0.00	2.84 ± 0.33
		12b	0.63 ± 0.04	2.97 ± 0.08
5.0	0.25	13	0.15 ± 0.04	4.55 ± 0.50
5.0	0.50	14a	0.32 ± 0.06	3.92 ± 0.71
		14b	0.40 ± 0.12	5.11 ± 0.63
5.0	0.75	15a	0.41 ± 0.11	3.51 ± 0.28
		15b	0.53 ± 0.09	4.94 ± 0.04
5.0	1.00	16a	0.49 ± 0.14	3.94 ± 0.52
		16b	0.59 ± 0.03	4.76 ± 0.11

[1] Top phase is designated by 'a' and bottom phase designated by 'b'. Phases without letter were stable single-phase β-glucan gum/WPI combinations.
[2] Mean ± standard deviation based on 4 determinations.

The variation in some of the results was relatively high due to the difficulty in the separation of individual phases for β-glucan and protein analysis. The relatively more viscous nature of the top phase resulted in some mixing of phases during separation using the separatory funnels. A better method for separation of phases is required for more accurate results. Successful determination of phase composition may indicate other combinations of β-glucan gum and WPI yielding stable single-phase systems.

Knowledge of stable single-phase combinations is critical for the successful production of beverages, since phase separation is detrimental to product appearance and quality as seen by consumers. Compatible concentrations and conditions for these ingredients are, therefore, needed in the formulation of food and beverage products.

3.3.4. Effect of pH/temperature treatments

The results for the processing treatment of β-glucan gum for 30 min at different pH/temperature conditions are shown in Table 3.5. The viscosity of β-glucan sols at pH 3 and 5, prior to heat treatment, was significantly lower ($p \leq 0.05$) than those at pH 7 and 9. These findings do not agree with those of Dawkins and Nnanna (1995), who found no pH effect on viscosity of oat β-glucan. Being a non-ionic polysaccharide, it is expected that β-glucan gum would be unaffected by pH. However, the behavior of residual proteins or other charged compounds present in the β-glucan gum may be altered by pH and have an effect on viscosity.

The β-glucan gum sols at pH 3, exhibited the largest decrease ($p \leq 0.05$) in

Table 3.5. Viscosity[1] of 0.5% (w/w) β-glucan gum sols at pH 3, 5, 7, and 9 after 30 min heat treatment at 55, 75 and 95 °C.

Temperature (°C)	pH	Viscosity[2,3] (mPa·s)
No treatment (Control)	3	4.80^{de}
	5	4.78^{de}
	7	5.04^{de}
	9	5.02^{abc}
55	3	4.62^{ef}
	5	4.76^{de}
	7	4.77^{de}
	9	4.85^{cd}
75	3	4.50^{f}
	5	4.77^{de}
	7	4.65^{ef}
	9	4.67^{def}
95	3	3.80^{g}
	5	4.99^{bc}
	7	5.18^{a}
	9	4.84^{cd}

[1] Means of 4 determinations are reported.
[2] Viscosity determined from the slope of the shear stress vs. shear rate curve.
[3] Means with different letters are significantly different ($p \leq 0.05$).

viscosity after heat treatment at 75 and 95°C for 30 minutes. The viscosity of pH 3 sols, processed at 75 and 95°C, were 4.50 and 3.80 mPa·s, respectively, compared to 4.80 mPa·s prior to heat treatment.

The β-glucan polymers are, therefore, susceptible to acid catalyzed hydrolysis. β-D-Glycosidic linkages, as found in β-glucan, are cleaved in acidic media, especially at elevated temperatures leading to a decrease in the MW of the polymer (Whistler and Daniel, 1985). With a decrease in MW, the internal friction or resistance to flow within the sol is decreased, producing the observed decrease in sol viscosity.

The inclusion of β-glucan gum in low pH foods, undergoing processing at elevated temperatures such as pasteurization or hot-fill processing, may not be desirable. Processing of such products may result in a decrease in viscosity and, therefore, a change in the functional properties of β-glucan gum within the product formulation. This may alter the product's sensory characteristics, resulting in detrimental effects on consumer acceptability. Thus, it is important to understand the extent of such changes so that adjustments can be made to the process and/or formulation to minimize such undesirable changes and produce an acceptable product.

3.4. CONCLUSIONS

The viscosity of β-glucan sols was significantly increased ($p \leq 0.05$) by the addition of sucrose and WPI. Rheological behavior, however, was not changed, remaining Newtonian in the shear rate and concentration ranges studied.

Spectrophotometer and DSC analyses demonstrated that the network formed by the β-glucan polymers may physically slow down the denaturation and aggregation of whey proteins. Increasing the ratio of β-glucan gum to WPI may produce larger enhancement in whey protein stability. Further investigation of the thermal properties of β-glucan gum/WPI sols may improve our understanding of thermal stabilization properties of β-glucan polymer network.

Both thermodynamically compatible and incompatible combinations of β-glucan gum and WPI were found. Examination of the protein and polysaccharide content of the separated phases was, however, inconclusive due to the difficulty in separating the phases for compositional analyses. Processing of β-glucan sols at low pH and elevated temperatures for 30 min resulted in a significant decrease ($p \leq 0.05$) in sol viscosity due to acid catalyzed hydrolysis of the β-glucan polymers. Determination of β-glucan gum functional properties and interactions with ingredients, such as WPI, are essential for formulation of stable food and beverage products.

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4. DEVELOPMENT OF A BARLEY β-GLUCAN BEVERAGE[1]

4.1. INTRODUCTION

Mixed-linkage (1→3), (1→4)-β-D-glucan (β-glucan) is a soluble fibre component found predominantly in barley and oats. β-Glucan has demonstrated health-promoting properties, when included in the diet. These include cholesterol lowering (Klopfenstein and Hoseney, 1987; Newman et al., 1992; Wang et al., 1992; Kahlon et al., 1993; Wang et al., 1997; Wood and Beer, 1998) and regulation of blood glucose levels (Wood, et al., 1990; Braaten et al, 1991; Wood et al., 1994).

It was the β-glucan in oats that earned Quaker Oats the right to label oatmeal, sold in the United States, as a "heart healthy" functional food (Klis, 1996). Barley or oat β-glucan concentrate may also be sold as a nutraceutical product. These two new product categories as outlined by Health Canada (1997), are currently not permitted to be labeled as such, with health promoting claims, according to Canadian legislation.

The nutritional benefits of β-glucan along with its functional properties, including thickening, stabilizing, emulsification and gelation, make it a favorable nutraceutical ingredient for inclusion in the formulation and production of functional foods and beverages (Dawkins and Nnanna, 1995; Burkus, 1996). β-Glucan has been isolated and concentrated from cereals using dry milling and sieving (Knuckles et al., 1992; Sunberg and Aman, 1994), dry milling and air

[1]A version of this chapter is to be submitted to Cereal Chemistry for consideration for publication.

classification (Wu et al., 1994) or alkali extraction procedures (Wood et al., 1989; Bhatty, 1993; Dawkins and Nnanna, 1993; Saulnier et al., 1994; Bhatty, 1995; Temelli, 1997). Food product applications of β-glucan gum or development of β-glucan enriched products, however, remain limited in both research and commercial settings.

Beverages are excellent carriers for ingredients with nutraceutical potential, such as soluble fibre or herbal extracts (Swientek, 1998, Pszczola, 1998). Being easily consumed along with a usual meal, a functional beverage (a beverage containing health-promoting nutraceutical ingredients) may enrich the meal and improve health. Barley β-glucan is particularly well suited for such an application, being capable of imparting a smooth mouthfeel to beverage products, while also making the beverage an excellent source of soluble dietary fiber. A barley β-glucan gum, having similar functionality, could potentially replace traditional beverage thickeners such as alginates, pectin, xanthan, and carboxymethylcellulose (Giese, 1992). However, there are no reports on the utilization of barley β-glucan as an ingredient in the production of a functional beverage product. Therefore, the objectives of this study were:

1. to develop a formulation and processing procedure for a functional beverage incorporating barley β-glucan,
2. to evaluate β-glucan beverage quality and acceptability in comparison to pectin using trained and consumer panel sensory evaluation techniques, and

3. to examine the shelf stability of β-glucan beverages using instrumental techniques.

4.2. MATERIALS AND METHODS

4.2.1. Materials

β-Glucan gum (85.63% w/w dry matter basis) was extracted from waxy, hulless barley (Bly Blend, mix of two experimental barley varieties, SB89528 and SB89497) at the POS Pilot Plant Corp. (Saskatoon, SK), according to Burkus and Temelli (1998), prior to this study. Lime peel pectin (Mexpectin RS 450, Grinsted, Denmark), sucrose, high fructose corn syrup (HFCS) (Isosweet 100, Staley, Decatur, IL), citric and ascorbic acids were obtained from a local food ingredient supplier (UFL Foods, Edmonton, AB). β-Carotene colorant was supplied by Hoffman-LaRoche (10% CWS β-carotene, Parsippany, NJ) and natural orange flavorings (Natural orange essence 3X enriched and cold-pressed Valencia terpeneless orange peel oil) were supplied by Firmenich (Safety Harbor, FL). Glass juice bottles (300 mL) were obtained from a local bottling company. Commercial products used in training sessions for the sensory panel were obtained from a local grocery store.

4.2.2. Beverage formulation and production

Beverage formulations (Table 4.1) were developed using laboratory-scale trials and bench top sensory evaluation by the research group. Focus group discussion was also conducted with 6 individuals, according to the methods outlined by Morgan (1988), for consumer assistance and product evaluation

Table 4.1. Formulations for β-glucan beverages.

Ingredients	Concentration (w/w)
Water	89.4, 89.2 or 89.0%[1]
β-Glucan Gum or Pectin	0.3, 0.5 or 0.7%
Sucrose	5.0%
High Fructose Corn Syrup	5.0%
Citric Acid	0.27%
Ascorbic Acid	0.03%
β-Carotene	10 ppm
Natural Orange Essence	0.01%
Terpeneless Orange Peel Oil	0.0005%

[1] 89.4, 89.2 and 89.0% water used for 0.3, 0.5, and 0.7% β-glucan or pectin, respectively.

during formulation development. Pilot plant production of the beverages was performed at the Food Processing Development Centre (Leduc, AB) according to the processing procedure developed during laboratory-scale trials (Figure 4.1). Following pasteurization, beverages were immediately hot filled into bottles, capped and placed into refrigerated storage after production.

Six beverage treatments were formulated with 0.3, 0.5 and 0.7% (w/w) β-glucan gum or pectin in triplicate. Pectin was selected for use in the control treatments since it is used quite extensively in beverage products. A total of 18 batches of beverages were produced in random order (20 L/batch).

4.2.3. Sensory evaluation

Standard sensory procedures were followed during recruitment, selection and training of sensory judges, 15 staff and students of the University of Alberta, and during trained and consumer panel evaluations (ASTM, 1979; Meilgaard et al., 1991). Panelists participating in the trained sensory panel were trained using repeated round table and individual evaluations of trial formulations of the pectin and β-glucan gum beverages, and reference samples.

Beverage and reference samples (25 mL) were presented to the trained sensory panel in capped glass jars, at 5°C. Samples were kept in a cold water bath to maintain serving temperature. Samples were presented according to a random order, balanced design along with 2 unsalted crackers and room temperature distilled water for rinsing, a napkin and score sheet on an off-white fibreglass tray. Panelists evaluated samples in standard sensory panel booths containing an

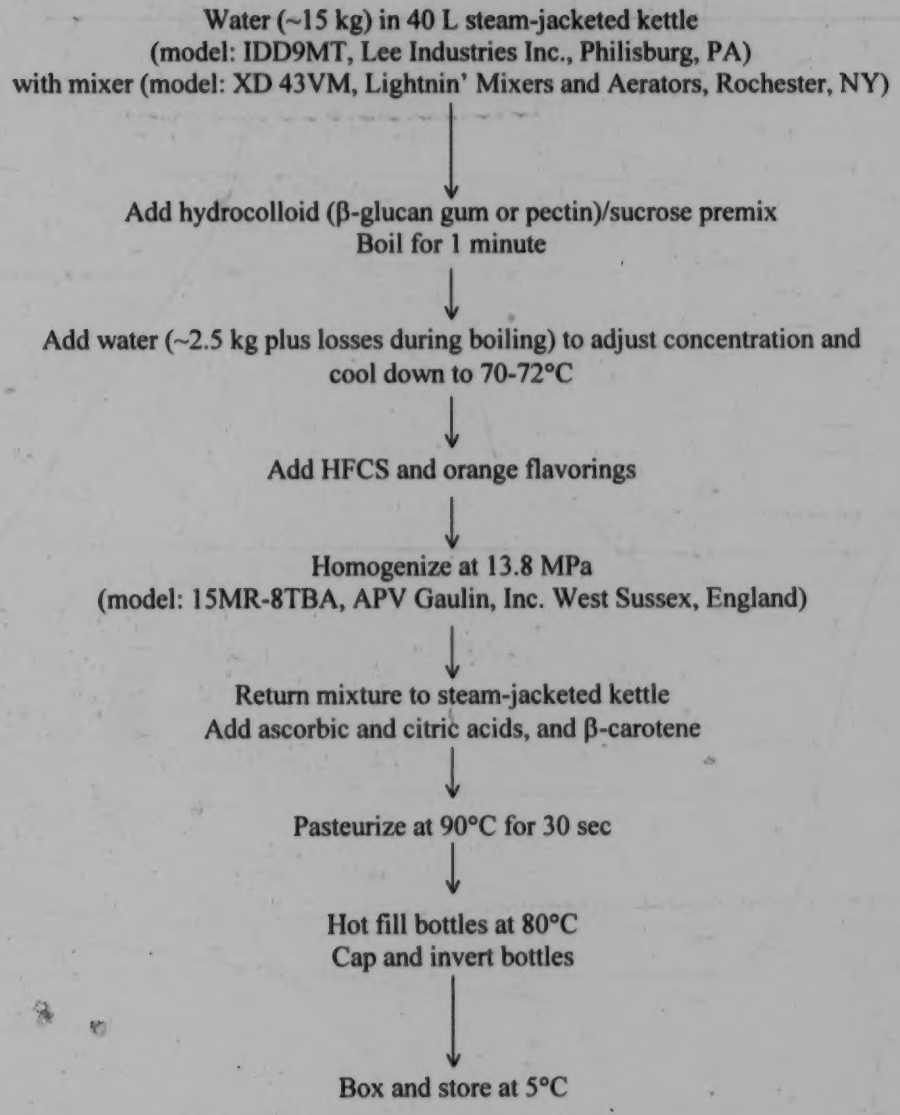
Water (~15 kg) in 40 L steam-jacketed kettle
(model: IDD9MT, Lee Industries Inc., Philisburg, PA)
with mixer (model: XD 43VM, Lightnin' Mixers and Aerators, Rochester, NY)
Add hydrocolloid (β-glucan gum or pectin)/sucrose premix
Boil for 1 minute
Add water (~2.5 kg plus losses during boiling) to adjust concentration and
cool down to 70-72°C
Add HFCS and orange flavorings
Homogenize at 13.8 MPa
(model: 15MR-8TBA, APV Gaulin, Inc. West Sussex, England)
Return mixture to steam-jacketed kettle
Add ascorbic and citric acids, and β-carotene
Pasteurize at 90°C for 30 sec
Hot fill bottles at 80°C
Cap and invert bottles
Box and store at 5°C

Figure 4.1. Pilot plant production of β-glucan and pectin beverages.

attribute definition sheet, stop watch and pencil. Panelists were rewarded for participation after each session.

Reference samples consisted of Minute Maid Original Orange Juice, Tang, and Lipton Orange Beverage, prepared according to directions on package, and a 0.45% (w/w) pectin solution. Sweetener, hydrocolloid, acid and flavor ingredients used in β-glucan beverage production were used to prepare solutions, as well as added to references to alter sample sensory attributes, for training purposes.

Trained panelists evaluated peely and fruity-orange aroma, sweetness and sourness intensity, and viscosity attributes for all 6 beverages. Panelists used a 15 cm line scale to evaluate product attributes. Attribute descriptors were presented at anchor points 1.25 cm from each end of line scale, which were none-extreme for aroma and taste intensity and not viscous-extremely viscous, for viscosity. Reference samples and scores, on 15 cm line, were provided for sweetness, sourness, and viscosity attributes (Table 4.2). Scores for reference samples were determined during training sessions (Meilgaard et al., 1991).

Table 4.2. Reference samples and scores for sweetness, sourness and viscosity.

Sample	Attribute	Score[1]
Tang	Sweetness	9.0
Minute Maid Orange Juice Original	Sourness	5.0
0.45% (w/w) Pectin	Viscosity	4.5

[1] Score on 15 cm line scale.

Consumer evaluation was performed at the University of Alberta fitness facility. Panelists, composed of 101 university students and staff, were provided 50 mL beverage samples in clear plastic cups, along with paper ballot, napkin, pencil, and distilled water in Styrofoam cup for rinsing on off-white fibreglass trays. Consumers were rewarded for participation.

Consumer panelists evaluated beverage sweetness, sourness, orange flavor, thickness and overall acceptability. Panelists used a nine-point hedonic scale with the categories: like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely, scoring 9 to 1, respectively, for evaluation of the beverages.

4.2.4. Shelf stability

β-Glucan and pectin beverages were stored, immediately following production, for 12 weeks at 5°C. Instrumental measures for examination of storage stability were performed weekly for 8 weeks and at 12 weeks. Beverage viscosity at 5°C was determined using a Haake rotational viscometer (RV-1 Rotational Viscometer, Haake, Germany). Two 8 mL samples of each beverage were used for viscosity determination. Product pH was obtained using a Corning pH meter (model 220, Corning Labware and Equipment, Corning, NY). Total aerobic plate counts were determined according to standard pour plate methods (Speck, 1979) on plate count agar (Bacto Plate Count Agar, DIFCO Laboratories. Detroit, MI) incubated at 37°C for 48 h. 'L', 'a' and 'b' color values were determined using a Hunter colorimeter (Hunter Associates Laboratory, Fairfax,

VA). Duplicate 5 g samples of each beverage were placed in a plastic petri dish and color was measured. Cloud stability was determined by measuring product absorbance with a spectrophotometer (HP 8452A, Hewlett Packard, Boise, ID) set at 660 nm (Versteeg et al., 1980). Duplicate measures were taken for all samples of the three replications produced.

4.2.5. Statistical analysis

Trained sensory evaluation was performed on all three replications. Consumers performed a single evaluation of 0.5% (w/w) pectin and β-glucan gum formulations. Instrumental measurements during storage stability study were performed in duplicate for all three replications of beverage production.

Analysis of variance of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 7 (SAS Institute, 1989). Multiple comparison of the means was performed by Student-Newman-Keuls (SNK) test, at α=0.05 level.

4.3. RESULTS AND DISCUSSION

4.3.1. Beverage formulation and production

The beverage formulation developed and used in pilot plant production is shown in Table 4.1. The production procedure is outlined in Figure 4.1. β-Glucan gum or pectin was premixed with sucrose to prevent clumping of the polysaccharide during addition to water. By such premixing, gum particles were separated thus leading to ease of mixing with water and faster processing.

The water added after the boiling step, served two purposes, to add volume withheld for cooling and account for water lost during boiling of mixture. Cooling by this method was necessary because the steam-jacketed kettle did not have simultaneous cooling capability. Acid ingredients and β-carotene were added as late as possible in the process to minimize β-carotene degradation and to minimize the exposure of β-glucan polymers to low pH at elevated temperatures. As shown earlier, the glycosidic linkages of the β-glucan polymer will undergo acid catalyzed hydrolysis at the elevated temperatures used during processing (section 3.3.4), such as pasteurization and hot-fill processing, and the low pH of the beverage. By minimizing this exposure, β-glucan polymers will be preserved and the viscosity maintained. Viscosity is important in the health promoting properties and functionality of β-glucan (Wang et al., 1992). This helps maintain potential health benefits, as well as, the body and mouthfeel of the beverages produced.

4.3.2. Sensory evaluation

The results of the trained panel sensory evaluation of barley β-glucan and pectin beverages are shown in Table 4.3. Peely and fruity-orange aroma and sweetness intensity of β-glucan and pectin beverages were similar ($p>0.05$). The type of hydrocolloid and concentration, therefore, did not affect these sensory attributes. No suppression of flavor intensity with increasing gum concentration was observed. This is contrary to what was expected since others have reported that with increasing hydrocolloid concentration the intensity of both basic tastes and aromatic flavors decreases (Vaisey et al., 1969; Moskowitz and Arabie, 1970;

Table 4.3. Mean sensory scores[1] for the sensory analysis of β-glucan and pectin beverages by trained panel.

Beverage	Peely Aroma[2]	Fruity-Orange Aroma[2]	Sweetness[2]	Sourness[2]	Viscosity[3]
0.3% β-Glucan	4.4 ± 3.2a	5.7 ± 3.5a	7.3 ± 3.6a	2.7 ± 1.4ab	2.8 ± 1.8e
0.5% β-Glucan	4.4 ± 3.3a	6.6 ± 4.2a	7.8 ± 3.4a	2.4 ± 1.4ab	4.1 ± 1.4c
0.7% β-Glucan	4.4 ± 3.2a	6.2 ± 3.6a	7.3 ± 3.4a	2.1 ± 1.1b	6.1 ± 1.9a
0.3% Pectin	4.9 ± 3.5a	6.1 ± 3.3a	7.1 ± 3.6a	3.0 ± 1.4a	2.6 ± 1.4e
0.5% Pectin	5.0 ± 3.1a	6.3 ± 3.7a	7.7 ± 3.6a	2.8 ± 1.7ab	3.4 ± 1.4d
0.7% Pectin	4.6 ± 3.7a	6.6 ± 3.9a	7.6 ± 3.7a	2.7 ± 1.4ab	4.7 ± 1.5b

[1] Means of 45 determinations (15 panelists x 3 replications) are reported.
[2] Anchor descriptors on 15 cm line scale: none-extreme
[3] Anchor descriptors on 15 cm line scale: not viscous-extremely viscous
a-e: Means ± standard deviation in columns with the same letter are not significantly different ($p>0.05$).

Pangborn et al., 1972; Pangborn and Szczesniak, 1974; Malkki et al., 1993). However, Pangborn et al. (1972) reported that a viscosity of approximately 16 mPa·s was required to significantly alter sweetness. All beverages, except the 0.7% β-glucan beverages had a viscosity of <16 mPa·s in this study. Malkki et al. (1993) also found that oat gum had less of an effect on taste intensities than other hydrocolloids. It is hypothesized, that the neutral charge of β-glucan presents less opportunity for binding of flavor components and, therefore, resulting in less sensory suppression. This explains the lack of flavor suppression seen with increasing concentrations for both hydrocolloids.

Beverage sourness intensity was, however, found to differ ($p \leq 0.05$) among beverages. Pectin beverages were slightly more sour than beverages formulated with β-glucan gum at each gum concentration, but this difference was not significant ($p > 0.05$). The pectin, originating from lime peels, contributes to the sour taste or acidity of the beverages due to the acidic nature of lime fruit it was extracted from. On the other hand, β-glucan sols are slightly alkaline (pH 7.6 for 1.0% β-glucan sol), since β-glucan is extracted from barley at alkaline conditions. Therefore, β-glucan gum does not contribute to beverage acidity and sourness intensity. In addition, there was a slight drop in sourness intensity with increasing gum concentration. The beverage containing 0.3% pectin, lowest viscosity, was significantly more sour ($p \leq 0.05$) than that with 0.7% β-glucan, highest viscosity. This is in agreement with the results of Pangborn et al. (1972), which showed that sourness, when compared to other tastes, was affected the most with increasing hydrocolloid concentration.

Trained panelists also determined that there were significant differences in the viscosity of the beverages (Table 4.3). As expected, the viscosity of beverages increased ($p \leq 0.05$) with gum concentration. Beverages containing 0.3% β-glucan gum and pectin had similar viscosity ($p > 0.05$). However, beverages containing 0.5 and 0.7% β-glucan were significantly more viscous ($p \leq 0.05$) than those with pectin at the same level. Less β-glucan gum is, therefore, required to produce beverages with similar viscosity to pectin, at concentrations ≥0.5% (w/w). β-Glucan beverages would then contain less soluble fibre than pectin beverages of the same viscosity. However, if more β-glucan gum is desired for a higher fibre claim but not the increase in viscosity, extraction conditions used for isolation of β-glucan from barley may be manipulated to obtain a lower viscosity β-glucan gum for the desired functionality (Burkus and Temelli, 1998). Thus, a high fibre, β-glucan beverage could be produced without significant increases in beverage viscosity, which might have adverse effects on consumer acceptability. However, it is currently not known if a change in health promoting properties will occur with the exchange of a small amount of high MW β-glucan for large amounts of low MW β-glucan.

The target market for a β-glucan functional beverage is composed of individuals who take an active role in their own health improvement, through dietary and lifestyle choices. They want a refreshing beverage that offers more than what is currently available in beverage products. The consumer evaluation was, therefore, performed at the University of Alberta physical fitness facility, assuming that individuals described by the target market would be found there.

The consumer panel found both 0.5% β-glucan and pectin beverages to be similar ($p>0.05$) in the acceptability of attributes evaluated (sweetness, sourness, orange flavor and thickness) and overall acceptability (Table 4.4). The 0.5% β-glucan and pectin beverages scored 6.3 and 6.7, respectively, on a nine point hedonic scale for overall acceptability. The frequency distribution plot for overall acceptability scores (Fig. 4.2), however, indicates that the majority of consumers participating in the study scored the beverages 7 and 8 out of 9. Therefore, the majority liked the beverages more than what is indicated by the mean score.

The similarity of the beverages shown by the consumer panel evaluation agrees with the lack of difference also found during trained panel evaluations. Although the trained panel found a significant difference ($p\leq0.05$) between the viscosity of 0.5% β-glucan and pectin beverages, this difference did not result in significantly different ($p>0.05$) acceptability scores for beverage viscosity.

4.3.3. Shelf stability

The 'L', 'a' and 'b' colorimeter values, measuring beverage white, red and yellow color components, respectively, for most β-glucan and pectin beverages decreased significantly ($p\leq0.05$) during the first week of storage (Table 4.5). After the first week, however, colorimeter values for all beverages, except the 'a' value for the 0.7% β-glucan beverage, stabilized ($p>0.05$). The decreases in colorimeter values observed during the first week may be due to the precipitation of insoluble material present in the beverages or changes in the β-carotene colorant. Visually, however, the changes indicated by colorimeter measurements did not appear to be noticeable.

Table 4.4. Mean sensory scores[1] for the consumer panel evaluation of 0.5% (w/w) β-glucan and pectin beverages.

Beverage	Sweetness	Sourness	Orange Flavor	Thickness	Overall Acceptability
β-Glucan	6.3 ± 1.7a	6.1 ± 1.6a	6.1 ± 1.7a	6.2 ± 1.9a	6.3 ± 1.8a
Pectin	6.7 ± 1.5a	6.3 ± 1.5a	6.5 ± 1.6a	6.4 ± 1.9a	6.7 ± 1.5a

[1] Mean score ± standard deviation on a nine point hedonic scale (1-dislike extremely, 9-like extremely). Means of 101 determinations reported.

a Means in the same column with the same letter are not significantly different ($p>0.05$).

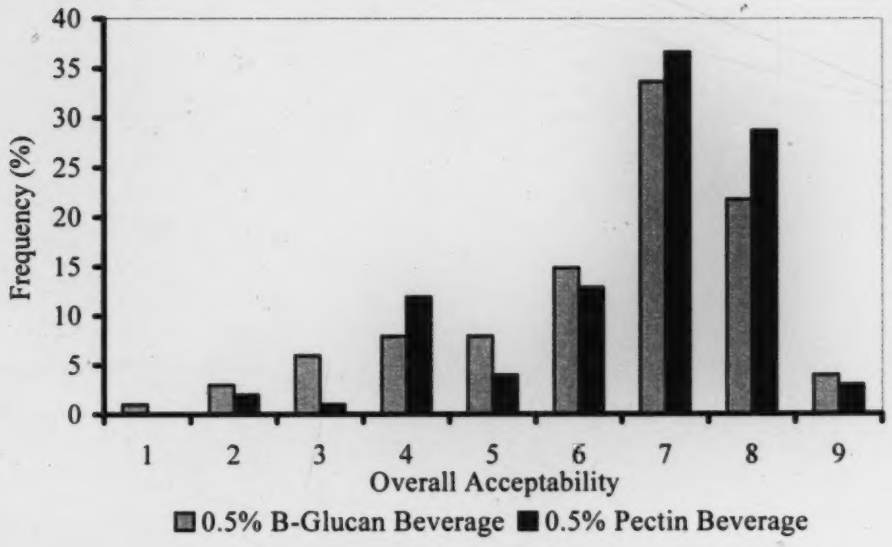

Figure 4.2. Frequency distribution of overall acceptability scores (on 9 point hedonic scale) for 0.5% (w/w) β-glucan and pectin beverages.

Table 4.5. Mean Hunter colorimeter values[1] for beverages, during 12-week refrigerated storage.

Gum	% (w/w)	Hunter Value	Week 0	1	2	3	5	6	7	8	12
Beta-Glucan	0.3	L	22.2a	19.3b	19.1b	19.1b	19.3b	19.1b	19.1b	18.9b	19.3b
		a	0.9a	1.3a	1.2a	1.1a	1.0a	0.9a	1.1a	0.8a	0.6a
		b	12.4a	9.3b	9.5b	9.4b	9.4b	9.3b	9.3b	9.1b	9.4b
Beta-Glucan	0.5	L	22.3a	19.8b	19.9b	20.0b	19.8b	20.0b	19.9b	19.8b	20.1b
		a	3.0a	1.2b	1.1b	0.9b	0.8b	0.7b	0.8b	0.7b	0.8b
		b	12.6a	9.8b	9.9b	9.9b	9.7b	9.9b	9.7b	9.6b	9.8b
Beta-Glucan	0.7	L	24.0a	20.3b	20.4b	20.3b	20.3b	20.4b	20.3b	20.4b	20.5b
		a	2.9a	1.3b	1.3bc	1.3bc	1.0d	1.0d	1.2bcd	1.2bcd	1.1cd
		b	13.6a	10.1b	10.2b	9.9b	10.1b	10.0b	10.0b	9.9b	10.0b
Pectin	0.3	L	21.8a	21.0b	21.0b	20.9b	20.9b	20.9b	21.1b	21.0b	21.0b
		a	-0.8a	-1.9b	-2.0b	-1.9b	-2.2b	-2.2b	-2.0b	-2.1b	-2.2b
		b	12.0a	11.0a	11.0a	10.9a	10.8a	10.8a	10.9a	10.9a	10.8a
Pectin	0.5	L	20.1a	21.3a	21.0a	21.1a	21.1a	20.8a	20.8a	20.7a	20.2a
		a	-0.5a	-1.3b	-1.7b	-1.7b	-2.0b	-2.1b	-1.9b	-2.0b	-2.3b
		b	10.4a	10.8a	11.0a	10.8a	10.7a	10.8a	10.7a	10.5a	10.1a
Pectin	0.7	L	20.1a	21.4b	21.3b	21.3b	21.3b	21.1b	21.1b	21.2b	21.1b
		a	-0.1a	-1.6b	-1.6b	-1.7b	-2.1b	-2.1b	-2.0b	-2.0b	-2.0b
		b	10.3a	11.2a	11.2a	11.0a	11.0a	10.9a	10.9a	10.9a	10.8a

[1] Means of 6 determinations are reported.

a-d: Means with different letters in rows are significantly different ($p<0.05$).

It is also important to note that the increasing levels of β-glucan gum affected the 'L' value, or beverage whiteness. Increased β-glucan gum concentration resulted in a lighter product ($p \leq 0.05$), which was cloudier in appearance. However, 'L' value was not affected by pectin concentrations ($p > 0.05$).

Beverage pH remained stable at 2.67-2.81 for all batches throughout the storage period. Total aerobic plate counts were also stable, showing no colony formation on initial plate counts and no microbial growth in the beverages throughout the storage period. The pasteurization treatment applied (90°C/30 sec) and the acidic nature of the beverage were adequate in preventing the growth of spoilage organisms, eliminating the need for the addition of preservatives. This keeps the ingredient list "natural", or free of "chemicals", which is considered desirable by many consumers.

Some of the beverages, especially those with a higher hydrocolloid content (0.7%, w/w) exhibited a slight decrease in viscosity throughout storage (Fig. 4.3). Viscosity was stable in all other formulations. β-Glucan gum was, therefore, stable within the low pH environment of a beverage formulation maintaining its consistency for 12 weeks. Any acid catalyzed hydrolysis of β-glucan polymers that may have occurred at elevated pasteurization and hot-filling temperatures did not continue during the refrigerated storage period. The order of beverages from least viscous to most viscous, as evaluated by the trained sensory panel (Table 4.3), agrees with instrumental measures of viscosity performed during the shelf stability evaluation.

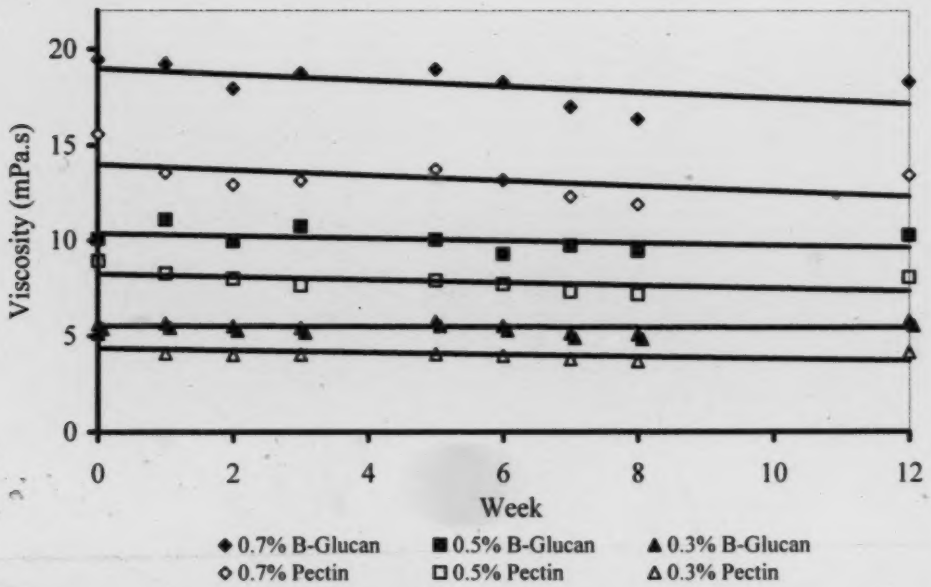

Figure 4.3. Viscosity of β-glucan and pectin beverages during 12-week storage. Means of 6 determinations are reported.

The β-glucan beverages exhibited cloud loss, measured as a decrease in absorbance at 660 nm, during the first 3 weeks (Fig. 4.4), but stabilized after week 3. The pectin beverages exhibited very little cloud loss. A small amount of precipitate was visible at the bottom of the β-glucan gum beverages, which was easily re-suspended when shaken. This precipitate was also visible in β-glucan sols in water and is, therefore, not due to precipitation caused by other ingredient interactions within the beverage formulation. The precipitate is assumed to be insoluble protein and fibre components present in the β-glucan gum at low levels. The insoluble material, being temporarily stabilized in the viscous network of the barley β-glucan sol, contributes to beverage cloudiness. The precipitation of this material is therefore a factor in the loss of beverage cloudiness. This may also explain the decreases observed in colorimeter values for beverages during the first week of storage.

The cloudiness of β-glucan beverages increased with increasing gum concentration. This increase, however, was not observed in the pectin beverages. β-Glucan gum may also, therefore, be useful as a clouding agent in functional beverage products. This is also supported by the increase in beverage whiteness, or 'L' value, observed with increasing concentrations of β-glucan gum.

4.4. CONCLUSIONS

Barley β-glucan gum was found to be stable within the acidic environment of an orange-flavored beverage, during processing and refrigerated storage. β-Glucan's ability to increase viscosity upon addition to water makes it an excellent

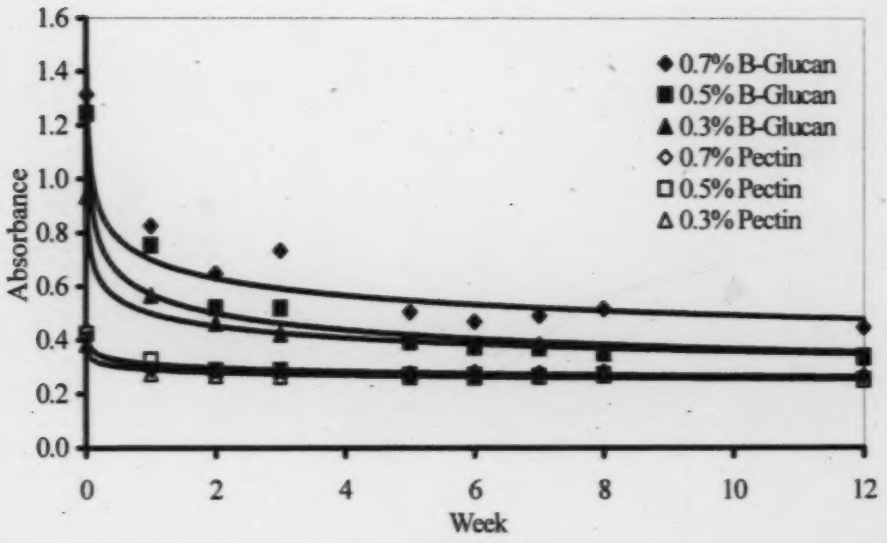

Figure 4.4. Absorbance values at 660 nm, as a measure of cloud stability of β-glucan and pectin beverages during 12-week storage. Means of 6 determinations are reported.

thickener for beverage applications. β-Glucan gum did not significantly alter the sensory attributes of beverage products or differ from pectin, except for sourness and viscosity. Increasing β-glucan gum was found to decrease sourness intensity scores, significantly at 0.7% when compared to 0.3% pectin, and increase beverage thickness more than pectin at concentrations ≥0.5%. Increasing β-glucan gum concentration also increased beverage cloud. An orange flavored β-glucan enriched beverage was successfully produced, found to be acceptable to consumers and stable over the 12-week storage period studied. β-Glucan, therefore, exhibits excellent potential as a nutraceutical ingredient for functional beverages, displaying both beneficial nutritional and physical functionality.

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5. DEVELOPMENT OF A BARLEY β-GLUCAN BEVERAGE WITH ADDED WHEY PROTEIN ISOLATE[1]

5.1. INTRODUCTION

There are new food products being developed and consumed that not only meet consumers' basic nutritional requirements, but possess medicinal qualities as well. The incorporation of nutraceutical ingredients into common food items, such as beverages, cereals and granola bars, transforms ordinary foods into functional foods and beverages. Two nutraceutical ingredients, that may find such functional food and beverage applications, are mixed linkage (1→3), (1→4)-β-D-glucan (β-glucan) from barley and whey protein isolate (WPI) from milk.

β-Glucan, the primary soluble fiber component of barley and oats, has exhibited hypocholesterolemic effects (Klopfenstein and Hoseney, 1987; Newman et al., 1992; Wang et al., 1992; Kahlon et al., 1993; Wang et al., 1997; Wood and Beer, 1998). Serum cholesterol reduction due to β-glucan consumption is attributed to two mechanisms. The viscous nature of β-glucan provides a physical barrier that inhibits the absorption of cholesterol and other lipid constituents and binds bile acids (cholesterol metabolite) in the gut. The unabsorbed and bound components then proceed to the large intestine and are excreted from the body. Some of the β-glucan that reaches the colon will also undergo fermentation by colonic microorganisms (Casterline et al., 1997; Wood and Beer, 1998; Bell et al., 1999). Fermentation of β-glucan results in the

[1]A version of this chapter is to be submitted to the Journal of Food Science for consideration for publication.

production of short chain fatty acids (SCFA). SCFA's, such as propionic and butyric acid, are then reabsorbed, enter circulation, and inhibit endogenous cholesterol synthesis.

β-Glucan has also been shown to slow glucose absorption and, therefore, regulate blood glucose (Wood, et al., 1990; Braaten et al, 1991; Wood et al., 1994). The viscous nature of β-glucan physically slows glucose absorption in the gut. This property may be useful in the production of foods for management of diabetes.

The exceptional nutritional quality of the whey proteins of milk has been known for quite some time (Holsinger et al., 1974). The anti-carcinogenic properties of whey have been shown by Bounous et al. (1991) and McIntosh et al. (1995). McIntosh et al. (1995) showed that dietary whey protein significantly ($p \leq 0.005$) decreases the incidence of chemically induced tumors in the rat colon. Whey protein enriched diets have also exhibited LDL cholesterol lowering and immune system stimulation effects (Bounous et al., 1988; Zhang and Beynen, 1993; Chinniah et al., 1997). Lactoferrin, lactoperoxidase, lysozyme and immunoglobulins, all minor whey protein components, have exhibited antimicrobial properties. Products, including baby food, chewing gum and mouthwash, that take advantage of these antimicrobial properties, are currently being developed (Jelen and Lutz, 1998).

Many whey beverages have been developed using both raw, unprocessed liquid whey, and whey protein concentrate and isolate powders (Holsinger et al., 1974; Pendergast, 1985). Whey protein is also finding its way on to the ingredient

lists of other foods, as producers realize its many nutritional and functional properties. On the other hand, there are no products rich in, or enriched with, β-glucan, other than products containing barley and oats. The incorporation of barley β-glucan and whey protein ingredients, which are low in a typical diet, into food products, may enrich the diet and aid in the prevention of certain diseases. There has been, however, no food products developed that take advantage of the potential of both these ingredients. There has also been little research into the behavior of β-glucan and WPI when used together in a food or beverage system.

Beverages are an excellent medium that may be used for the addition of nutraceutical components for enrichment of the diet (Kuhn, 1998). A beverage may be consumed along with a typical meal and, as a result, enrich the nutritional value of the meal without altering a consumer's dietary habits. Although, there have been numerous whey beverages developed, none have the added benefits of β-glucan soluble fibre. The objectives of this study were, therefore,

1. to develop a formulation and processing procedure for a functional beverage incorporating the barley β-glucan and WPI,
2. to evaluate beverage quality and acceptability using trained and consumer panel sensory evaluation techniques and,
3. to examine the shelf stability of β-glucan/WPI beverages using instrumental techniques.

5.2. MATERIALS AND METHODS

5.2.1. Materials

β-Glucan gum, (85.63% w/w, dry matter basis) was extracted from waxy, hulless barley at the POS Pilot Plant Corp. (Saskatoon, SK), according to Burkus and Temelli (1998), prior to this study. Alacen 895 whey protein isolate (WPI), >90% protein, was supplied by New Zealand Milk Products (Santa Rosa, CA). Sucrose, high fructose corn syrup (HFCS) (Isosweet 100, Staley, Decatur, IL), citric and ascorbic acids were obtained from a local food ingredient supplier (UFL Foods, Edmonton, AB). β-Carotene colorant was supplied by Hoffman-LaRoche (10% CWS β-carotene, Parsippany, NJ) and natural orange flavorings (Natural orange essence 3X enriched and cold-pressed Valencia terpeneless orange peel oil) were supplied by Firmenich (Safety Harbor, FL). Glass juice bottles (300 mL) were obtained from a local bottling company. An anti-foaming agent, Atmos 300 (Atkemix, Inc., Brantford, ON), was also added to eliminate foaming during the production of the beverage. Commercial products used in sensory panel training sessions were purchased at a local grocery store.

5.2.2. Beverage formulation and production

Beverage formulations (Table 5.1) were developed using laboratory-scale trials and bench top sensory evaluation by the research group. Pilot plant production of the beverage was performed at the Food Processing Development Centre (Leduc, AB) according to the processing procedure developed during laboratory-scale trials (Fig. 5.1).

Table 5.1. Formulations for β-glucan/WPI beverages.

Ingredients	Concentration (w/w)
Water	89.2, 88.7, 88.2 or 87.7%[1]
β-Glucan Gum	0.5%
WPI	0, 0.5, 1.0 or 1.5%
Sucrose	5.0%
High Fructose Corn Syrup	5.0%
Citric Acid	0.27%
Ascorbic Acid	0.03%
β-Carotene	10 ppm
Natural Orange Essence	0.01%
Terpeneless Orange Peel Oil	0.0005%
Antifoaming agent	0.005%

[1] 89.2, 88.7, 88.2 or 87.7% water used for 0.5% β-glucan gum beverages with 0, 0.5, 1.0 and 1.5% WPI, respectively.

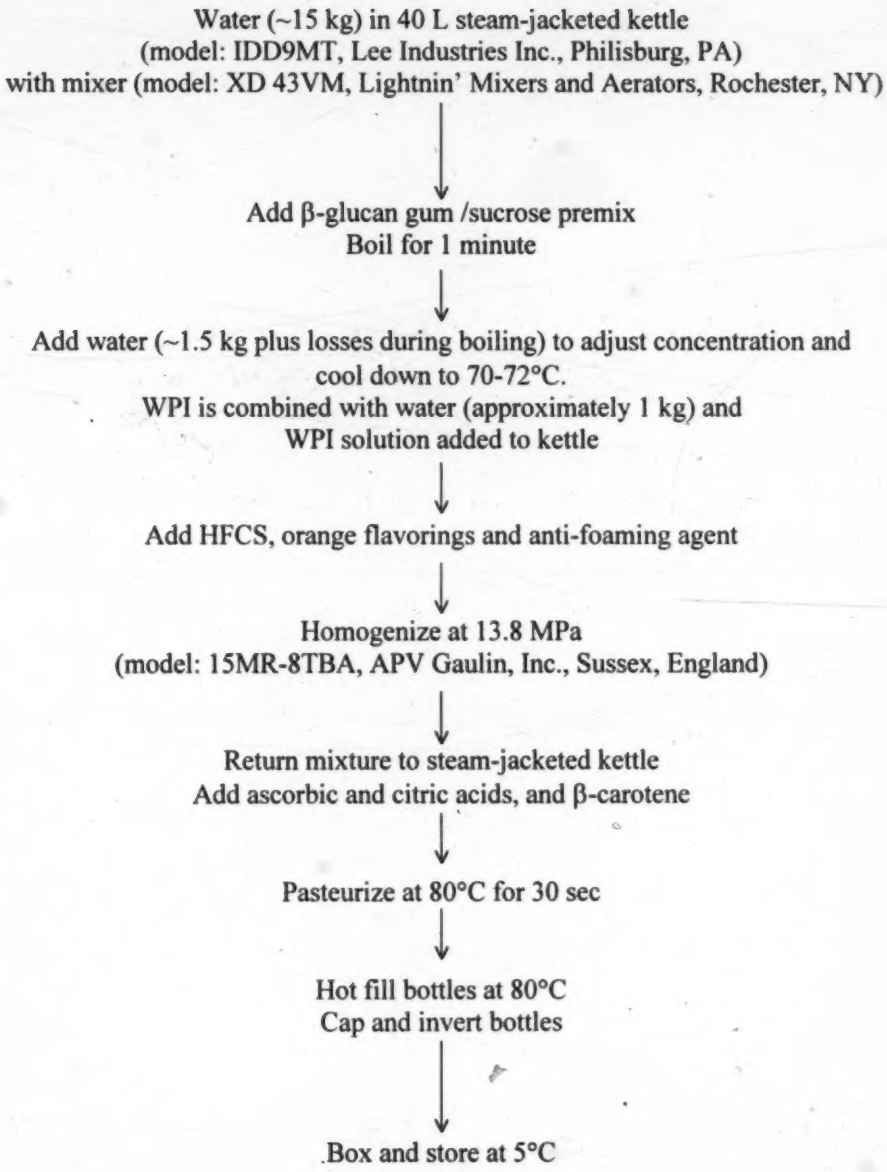
Water (~15 kg) in 40 L steam-jacketed kettle
(model: IDD9MT, Lee Industries Inc., Philisburg, PA)
with mixer (model: XD 43VM, Lightnin' Mixers and Aerators, Rochester, NY)
Add β-glucan gum /sucrose premix
Boil for 1 minute
Add water (~1.5 kg plus losses during boiling) to adjust concentration and
cool down to 70-72°C.
WPI is combined with water (approximately 1 kg) and
WPI solution added to kettle
Add HFCS, orange flavorings and anti-foaming agent
Homogenize at 13.8 MPa
(model: 15MR-8TBA, APV Gaulin, Inc., Sussex, England)
Return mixture to steam-jacketed kettle
Add ascorbic and citric acids, and β-carotene
Pasteurize at 80°C for 30 sec
Hot fill bottles at 80°C
Cap and invert bottles
Box and store at 5°C

Figure 5.1. Process for the pilot plant production of β-glucan/WPI beverages.

Experimental design consisted of four beverage treatments formulated with 0.5% (w/w) β-glucan gum and 0.0, 0.5, 1.0 or 1.5% (w/w) WPI and replicated in triplicate. A total of 12 batches of beverages were produced in random order. Each batch was approximately 20 L. Beverages were immediately bottled, capped and placed into refrigerated storage after production.

5.2.3. Sensory evaluation

Standard sensory procedures were followed during recruitment, selection and training of sensory judges, 10 staff and students of the University of Alberta, and during trained and consumer panel evaluations (ASTM, 1979; Meilgaard et al., 1991). Panelists participating in the trained sensory panel were trained using repeated round table and individual evaluations of reference samples, with and without addition of ingredients to alter sample sensory attributes, and trial formulations of the WPI/β-glucan beverages.

Beverage and reference samples (25 mL) were presented to the trained sensory panel, in 30 mL plastic cups with lids, at 5°C. Samples for sensory evaluation were kept in a cold water bath to maintain serving temperature. Samples were presented according to random, balanced design along with a 1 oz. plastic cup containing 2 unsalted crackers, a glass of room temperature distilled water for rinsing the palate in between samples, a napkin and score sheet on an off-white fibreglass tray. Panelists evaluated samples in standard sensory panel booths containing an attribute definition sheet and pencil. Panelists were rewarded for participation after each session.

Reference samples included, Minute Maid Original Orange Juice, Tang, Tropicana Orange Juice, prepared according to the directions on the package, and a 1.0% (w/w) WPI solution and a 0.45% (w/w) pectin solution. WPI, sweetener, hydrocolloid, acid and flavor ingredients for beverage production were also used to prepare solutions, and added to references to alter sample sensory attributes, for training purposes.

Trained panelists evaluated beverage cloudiness, sweetness, sourness, orange flavor and whey flavor intensity, and viscosity. Panelists used a 15 cm line scale to evaluate product attributes. Attribute descriptors were presented at anchor points 1.25 cm from each end of line scale. Anchor points were labeled none to extreme at left and right, respectively, for sweetness, sourness, orange flavor and viscosity attributes. Cloudiness intensity left and right anchor points were labeled low and extreme, whereas those for whey flavor intensity were labeled weak and strong, respectively. Reference samples and scores, on 15 cm line, were provided for sweetness, sourness, whey flavor and viscosity attributes (Table 5.2). Scores for reference samples were determined during training sessions (Meilgaard et al., 1991).

Table 5.2. Reference samples and scores for sweetness, sourness and viscosity.

Sample	Attribute	Score[1]
Tang	Sweetness	9.0
Minute Maid Orange Juice Original	Sourness	5.0
1.0% (w/w) WPI	Whey Flavor	7.5
0.45% (w/w) Pectin	Viscosity	4.5

[1] Score on 15 cm line scale.

Consumer evaluation was performed at the University of Alberta fitness facility. Panelists, composed of 76 university students and staff, were provided 50 mL beverage samples in clear plastic cups, along with a paper ballot, napkin, pencil, and distilled water in Styrofoam cup for rinsing, on off-white fibreglass trays. Consumers were rewarded for participation.

Consumer panelists evaluated beverage appearance, sweetness, sourness, orange flavor, thickness and overall acceptability. Panelists used a nine-point hedonic scale, with the categories: like extremely, like very much, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, dislike very much and dislike extremely, scoring 9 to 1, respectively, for evaluation of the beverages.

5.2.4. Shelf stability

β-Glucan/WPI beverages were stored, immediately following production, for 8 weeks at 5°C. Instrumental measures for examination of storage stability were performed weekly. Beverage viscosity at 5°C was determined using a Haake rotational viscometer (RV-1 Rotational Viscometer, Haake, Germany). Two 8 mL samples of each beverage were used for viscosity determination. Product pH was obtained using a Corning pH meter (model 220, Corning Labware and Equipment, Corning, NY). Total aerobic plate count was determined according to standard pour plate methods (Speck, 1979) on plate count agar (Bacto Plate Count Agar, DIFCO Laboratories, Detroit, MI) incubated at 37°C for 48 h. 'L', 'a' and 'b' color values were determined using a Hunter Colorimeter (Hunter Associates Laboratory, Fairfax, VA). Duplicate samples of 5 g of each beverage were placed

in a plastic petri dish for color measurement. Cloud stability was determined by measuring product absorbance with a spectrophotometer (HP 8452A, Hewlett Packard, Boise, ID) set at 660 nm (Versteeg et al., 1980). Duplicate measures were taken for all samples of the three replications produced.

5.2.5. Statistical Analysis

Trained sensory evaluation was performed on all three replications. Consumers evaluated all four β-glucan/WPI formulations. Instrumental measurements during storage stability study were performed in duplicate for all three replications of beverage production.

Analysis of variance of the results was performed using General Linear Model procedure of SAS Statistical Software, Version 7 (SAS Institute, 1989). Multiple comparison of the means was performed by Student-Newman-Keuls (SNK) test, at α=0.05 level.

5.3. RESULTS AND DISCUSSION

5.3.1. Beverage formulation and production

The formulation developed for 0.5% β-glucan beverages with and without WPI is shown in Table 5.1. A concentration of 0.5% β-glucan gum was chosen because it proved to be easier to work with during production (faster dispersion than 0.7% β-glucan gum) of beverages without WPI, resulted in an acceptable product (Chapter 4) and provided the β-glucan/WPI beverage with >0.75 g soluble fibre/serving, the requirement of the oat β-glucan health claim (Klis, 1996). The beverage production procedure for the pilot plant was developed at

laboratory scale and is outlined in Figure 5.1. An antifoaming agent was added to the formulation during production at pilot plant scale. This was necessary to minimize foaming since the increased air incorporation resulted in foam development during mixing of beverage ingredients with pilot plant equipment, which required high shear rates. In addition, the presence of β-glucan gum increases the stability of whey protein foams (Burkus and Temelli, 1999). Addition of an anti-foaming agent minimized any potential losses or undesired effects due to uncontrolled foam production.

It was necessary to retain a portion of the water, approximately 2.5 kg, 0.5 - 1.0 kg for pre-hydration of WPI and the remaining for rapid cooling of the mixture from β-glucan gum hydration temperature. Manual prehydration of WPI in a small portion of water, before adding to the kettle, made complete mixing without lumping possible in a relatively short period of time.

Pasteurization was performed at 80°C for 30 sec. This is 10°C lower than the pasteurization treatment used in the production of beverages without added WPI (Chapter 4). This temperature was used to minimize denaturation of the heat sensitive whey proteins. At pH 3.0, the approximate pH of the beverages, whey protein denaturation was delayed until a temperature of 87°C, determined using differential scanning calorimetry (DSC) (Fig. 3.4). This is a large improvement in thermal stability, since denaturation of whey proteins begins at 77°C at pH 7.0 (Fig. 3.5). β-Glucan addition also was found to slightly increase WPI heat stability (Figs. 3.4 and 3.5). By applying temperatures below 87°C, the WPI in the beverages would maintain its native form. This minimizes, or eliminates, any

potential precipitation due to heat induced protein denaturation and aggregation and preserves the original functionality of the WPI, such as solubility and thickening properties.

5.3.2. Sensory evaluation

The results of the trained panel sensory evaluation of the beverages are shown in Table 5.3. WPI was found to significantly increase ($p \leq 0.05$) the cloudiness of the beverages produced. At 0.5 and 1.0% (w/w) WPI, beverage cloudiness scores were approximately double and significantly higher ($p \leq 0.05$) than that of the beverage without WPI. The beverage with 1.5% (w/w) WPI was significantly more cloudy ($p \leq 0.05$) in appearance than all other beverages with and without WPI.

Beverages were similar in sweetness intensity ($p > 0.05$). The addition of WPI did not alter the sweetness of the beverages, even though the sweetness score was slightly higher at 1.5% WPI addition. Less sweetener may be required, when higher concentrations of WPI are used, to achieve similar sweetness intensity. Sourness intensity, however, was significantly reduced by the addition of WPI ($p < 0.05$). As WPI concentration increased, sourness intensity decreased.

Orange flavor intensity also significantly decreased ($p \leq 0.05$) with the addition of WPI. The formulations containing 0.5 and 1.0% WPI also differed significantly ($p \leq 0.05$) in orange flavor intensity. However, the beverage containing 1.5% WPI scored slightly higher orange flavor intensity than the 1.0% WPI formulation (7.4 and 6.6, respectively).

Table 5.3. Trained panel sensory evaluation results[1] for 0.5% (w/w) β-glucan beverages with and without WPI.

% WPI (w/w)	Cloudiness[2]	Sweetness[3]	Sourness[3]	Orange[3] Flavor	Whey[4] Flavor	Viscosity[3]
0	5.2 ± 3.1c	7.2 ± 2.0a	4.0 ± 1.3a	9.0 ± 2.0a	2.7 ± 1.9b	2.5 ± 1.2c
0.5	10.4 ± 1.7b	7.2 ± 2.3a	3.2 ± 1.2b	7.7 ± 2.0b	4.8 ± 2.6a	3.9 ± 1.6b
1.0	10.3 ± 2.1b	6.7 ± 2.1a	2.9 ± 1.3bc	6.6 ± 2.5c	4.9 ± 1.9a	3.8 ± 1.5b
1.5	11.8 ± 1.9a	7.5 ± 2.6a	2.4 ± 0.9c	7.4 ± 2.2bc	5.7 ± 2.1a	4.8 ± 2.0a

[1] Means of 30 determinations (10 panelists x 3 replications) are reported.
[2] Anchor descriptors on 15 cm line scale: low-extreme
[3] Anchor descriptors on 15 cm line scale: none-extreme
[4] Anchor descriptors on 15 cm line scale: weak-strong
a-c: Means ± standard deviation with different letters in each column are significantly different ($p<0.05$).

WPI imparted a specific whey flavor to the beverages. Among the three beverages containing WPI there was no difference in whey flavor intensity ($p>0.05$), although, the whey flavor intensity increased slightly with increasing WPI concentration. Panelists also scored the beverage without WPI as having a slight whey flavor. The panel, therefore, must have included some flavor characteristics of other ingredients, common to all four formulations, into the whey flavor attribute. These other flavors may have resulted in a slight increase in whey flavor scores for beverages with WPI.

Viscosity scores were also significantly increased ($p\leq0.05$) with the addition of WPI to the 0.5% β-glucan beverage formulation. At concentrations of 0.5 and 1.0% WPI, viscosity scores of 3.9 and 3.8, respectively, did not differ significantly ($p>0.05$). However, at 1.5% WPI, the viscosity score increased to 4.8, which was a significant increase over the other beverages. WPI, therefore, did contribute to thickening effect along with that of the β-glucan gum.

The results of the consumer evaluation of beverages are shown in Table 5.4. The consumer panel found no significant differences ($p>0.05$), in the degree of liking, for all attributes studied, with the addition of WPI to the 0.5% (w/w) β-glucan beverage. There was, however, a slight decreasing trend in consumer acceptance for appearance, sourness, orange flavor, thickness and overall acceptability attributes, with the addition of WPI. Consumer results for sweetness indicate that with increased WPI the sweetness acceptability score increased slightly. According to the trained panel results (Table 5.3), WPI addition increased beverage sweetness slightly. Consumers may, therefore, prefer slightly

Table 5.4. Results of consumer panel sensory evaluation of 0.5% (w/w) β-glucan beverages with and without WPI, mean score on 9 point hedonic scale[1].

% WPI (w/w)	Appearance	Sweetness	Sourness	Orange Flavor	Thickness	Overall Acceptability
0	7.0 ± 1.6a	6.3 ± 1.8a	6.0 ± 1.7a	6.2 ± 2.0a	6.3 ± 1.9a	6.1 ± 1.9a
0.5	6.6 ± 1.4a	6.4 ± 1.7a	5.9 ± 1.5a	6.0 ± 1.7a	6.0 ± 1.8a	6.1 ± 1.6a
1.0	6.5 ± 1.5a	6.4 ± 1.8a	5.7 ± 1.6a	5.9 ± 1.9a	6.0 ± 1.8a	6.1 ± 1.9a
1.5	6.5 ± 1.5a	6.5 ± 1.7a	5.8 ± 1.8a	6.1 ± 1.9a	5.8 ± 2.2a	6.0 ± 2.0a

[1] Means of 76 determinations are reported.

a: Means ± standard deviation with the same letter in each column are not significantly different ($p>0.05$).

sweeter beverage formulations, as found with WPI addition to the β-glucan beverage. The consumer trend for sourness, along with trained panel results, also suggests that consumers may prefer the more sour taste of the beverage without or with lower concentrations of WPI. Additional citric and ascorbic acid could be added to formulations with higher WPI concentrations to achieve sourness levels similar to formulations without WPI. It is important to note that the frequency distribution of overall acceptability scores for the beverages (Fig. 5.2) indicates that the majority of consumers, scoring the beverages 7 and 8 out of 9, liked the beverages more than that indicated by the mean scores (Table 5.4).

5.3.3. Shelf stability

Change in beverage cloud stability over the 8 week refrigerated storage period, indicated by absorbance at 660 nm, is shown in Figure 5.3. Beverages higher in WPI concentration exhibited the greatest cloud loss. The beverage without WPI showed the greatest cloud stability with the least decrease in absorbance. Overall, beverages showed significant decreases ($p \leq 0.05$) in absorbance during the first two weeks of storage. After week 2, however, no further significant changes ($p > 0.05$) in product absorbance were observed. A cream colored, liquid phase was observed at the bottom of the beverages containing WPI. The WPI appears also to have caused the precipitation of some other components that contribute to cloud into the bottom portion of the beverages. This separation is due to the incompatibility of β-glucan polysaccharide and whey proteins as discussed in Chapter 3. Beverages with

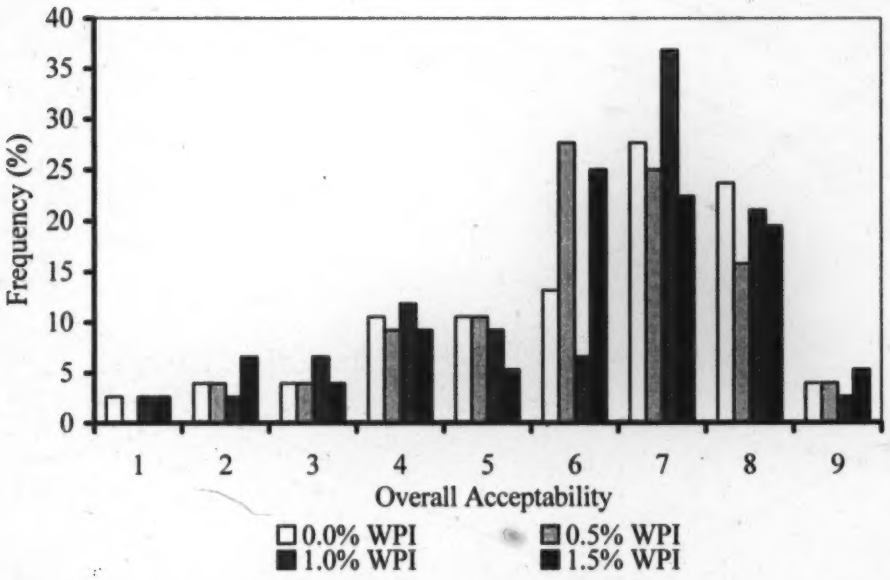

Figure 5.2. Frequency distribution of overall acceptability scores (on 9-point hedonic scale) for 0.5% (w/w) β-glucan beverages with and without WPI.

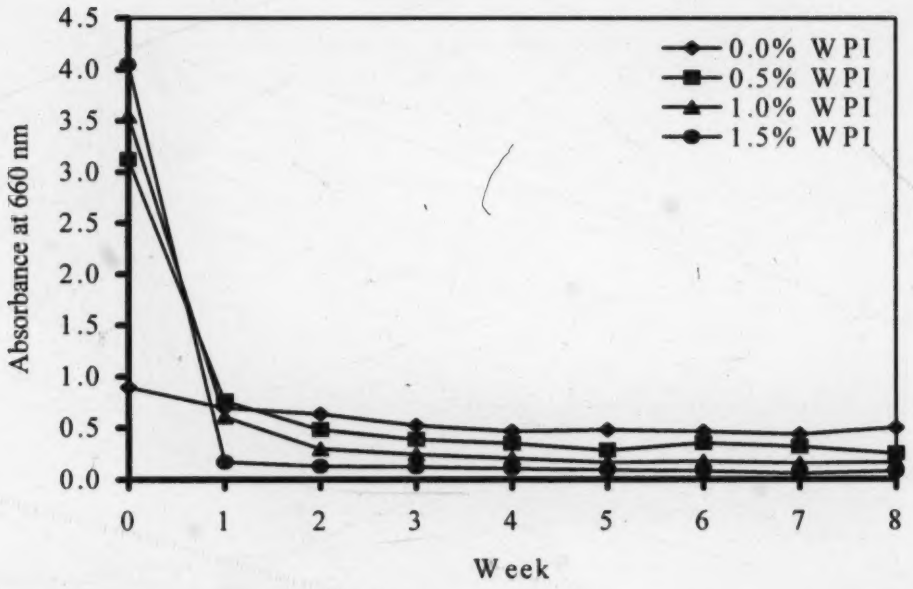

Figure 5.3. Cloud stability (absorbance at 660 nm) of 0.5% (w/w) β-glucan beverages with and without WPI, during 8 week refrigerated storage. Means of 6 determinations are reported.

highest WPI showed the greatest decrease in cloud. The beverage without WPI showed the greatest absorbance, or cloud, after 2 weeks of storage, followed by formulations with 0.5, 1.0 and 1.5% (w/w) WPI. The beverages, however, easily returned to a single-phase mixture upon shaking of the bottle.

Hunter color results indicate no significant changes ($p>0.05$) in 'L' and 'b' values, white and yellow components of color, respectively, during the 8-week storage period, for all beverages (Table 5.5). The 'a' value, however, for the beverage containing 1.0% WPI decreased significantly ($p\leq0.05$), from 2.0 to 1.7, at week 0 and 8, respectively. This indicates a decrease in the red intensity of this beverage during storage.

The addition of 0.5 and 1.0% WPI to the 0.5% β-glucan gum beverage resulted in a significant increase ($p\leq0.05$) in the 'L' value. However, the 0.5 and 1.0% formulations were similar ($p>0.05$). The addition of 1.5% WPI resulted in a significant increase ($p\leq0.05$) above all other beverage 'L' values. Similar results were observed for 'a' values, where 0.5 and 1.0% WPI beverages were more red than the formulation without WPI and the 1.5% WPI product had the highest 'a' value. All three beverages differed significantly ($p\leq0.05$) in 'b' value, which increased with WPI addition.

Beverage viscosity was stable during the storage period (Fig. 5.4.). No significant decreases ($p>0.05$) in viscosity were observed. Any acid-catalyzed hydrolysis of β-glucan that may have occurred at the elevated temperatures of pasteurization and hot-fill processing did not continue during storage. However, there were some difficulties in measuring beverage viscosity. The shear rates

Table 5.5. Mean Hunter colorimeter values[1] for beta-glucan beverages with and without WPI, during 8-week refrigerated storage.

WPI		Week								
% (w/w)	Hunter Value	0	1	2	3	4	5	6	7	8
0.0	L	20.7a	20.6a	20.5a	20.6a	20.4a	21.9a	20.9a	21.0a	20.6a
	a	0.6a	0.6a	0.7a	0.6a	0.6a	0.6a	0.5a	0.5a	0.5a
	b	9.7a	9.9a	9.8a	9.9a	9.9a	9.3a	9.5a	9.5a	9.7a
0.5	L	32.3a	32.0a	31.9a	32.7a	32.2a	30.7a	32.2a	32.9a	32.2a
	a	1.9a	1.9a	1.9a	1.7a	2.1a	1.8a	2.0a	1.7a	1.8a
	b	13.2a	13.2a	13.2a	12.4a	13.3a	13.0a	13.2a	12.9a	13.3a
1.0	L	32.2a	31.7a	31.6a	31.6a	31.7a	32.2a	32.1a	32.6a	31.7a
	a	2.0a	2.0a	2.0a	2.0a	1.9ab	1.8ab	1.8ab	1.8ab	1.7b
	b	12.6a	12.8a	12.8a	12.8a	12.7a	12.5a	12.6a	12.5a	12.8a
1.5	L	35.9a	35.5a	35.5a	35.6a	36.1a	36.2a	36.0a	36.0a	35.8a
	a	3.6a	3.6a	3.6a	3.6a	3.5a	3.5a	3.3a	3.3a	3.4a
	b	14.2a	14.4a	14.5a	14.4a	14.2a	14.3a	14.2a	14.3a	14.6a

[1] Means of 6 determinations are reported.

a-b: Means with different letters in the same row are significantly different ($p<0.05$).

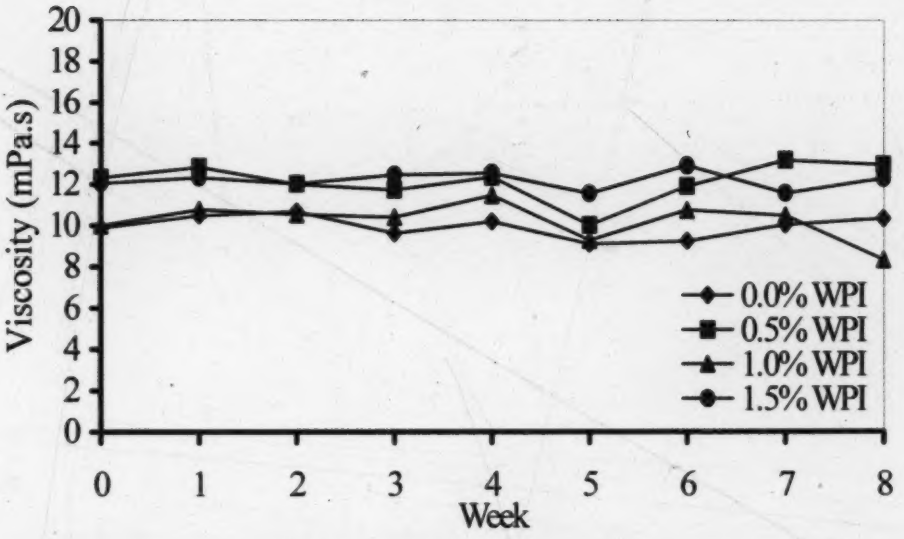

Figure 5.4. Viscosity of 0.5% (w/w) β-glucan beverages, with and without WPI, during 8-week refrigerated storage. Means of 6 determinations are reported.

required for measurement of beverage viscosity resulted in foam formation with the WPI beverages. This resulted in the variability observed in the viscosity data for shelf stability analysis.

The pH of beverages was found to significantly increase ($p \leq 0.05$) with the addition of WPI. The 0.5% (w/w) β-glucan gum beverage, without WPI, maintained an average pH of 2.60 ± 0.02 (mean ± standard deviation) during the storage period. Beverage pH increased to 2.91 ± 0.03, 3.16 ± 0.01 and 3.36 ± 0.01 with the addition of 0.5, 1.0 and 1.5% (w/w) WPI, respectively. Beverage pH remained stable for all formulations during the 8-week refrigerated storage period. The WPI, acting as a buffer, reduced acidity as indicated by the pH increase observed. Sourness intensity scores, determined by the trained sensory panel (Table 5.3), were also decreased as a result of WPI addition to the β-glucan gum beverage.

Beverages with WPI appeared to have some microbiological contamination whereas the beverage without WPI exhibited no growth. Beverages with WPI had mean total plate counts of 1, 15 and 71 colony forming units (CFU)/mL for 0.5, 1.0 and 1.5% WPI, respectively. Not all batches of individual formulations exhibited the same level of growth. For example, one batch of beverages containing 1.5% WPI had total plate counts of >200 CFU/mL, while the other batches had counts of 2 and 10 CFU/mL. The source of contamination is unknown. A WPI solution plated on the same media used for the total plate counts resulted in no growth. There is, therefore, no reason to suspect the WPI as the source of contamination. The pH increase seen in formulations with added WPI

could have resulted in a less effective pasteurization treatment, leading to a protective effect on contaminating microorganisms originating from other ingredients, such as the β-glucan gum, which did show microbial growth when plated. Such a protective effect of WPI would explain why no growth was observed in the 0.5% β-glucan gum beverage without WPI, where the pH/temperature/time combination used in pasteurization was effective.

5.4. CONCLUSIONS

An orange-flavored beverage enriched in barley β-glucan and WPI was produced at the pilot plant level. WPI was found to have significant effects on the cloudiness, sourness, orange and whey flavor intensity and viscosity of 0.5% β-glucan beverage, as determined by a trained sensory panel. Sweetness was not significantly changed. Consumers also found no significant differences among beverages. The trends observed in the consumer results, however, indicate that with increases in WPI concentration the degree of consumer liking decreased slightly. The beverages were found to be stable during the 8-week refrigerated storage period. Phase separation did occur, especially in the beverages formulated with higher concentrations of WPI. The phases were easily mixed upon shaking, forming a homogenous beverage. Although, some phase separation was observed in the beverages with WPI, functional beverages were produced which consumers found to be acceptable and have the potential health-enhancing properties of both barley β-glucan and whey proteins.

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6. CONCLUSIONS AND RECOMMENDATIONS

β-Glucan soluble fibre has exhibited many beneficial functional and health-enhancing properties. These properties have generated much research interest in the extraction and characterization of β-glucan from barley and oats. Product applications of β-glucan, however, remain limited. Barley, one of the most widely grown crops in Canada, being exceeded in production only by wheat, is an excellent starting material for the preparation of β-glucan gum extracts.

β-Glucan gum, when mixed with water, forms a viscous hydrocolloid sol. The 0.5 and 1.0% (w/w) β-glucan sols studied were found to have a viscosity of 8.25 and 26.14 mPa·s at 5°C, and 4.41 and 11.56 mPa·s at 25°C, respectively. Both sols exhibited Newtonian behavior for the shear rates selected, at both temperatures. Knowledge of a gum's rheological behavior at different temperatures is essential for product applications differing in serving temperature. The addition of sucrose and WPI significantly increased ($p \leq 0.05$) the viscosity of 0.5 and 1.0% (w/w) β-glucan sols. Rheological behavior, however, remained Newtonian. Interactions between β-glucan polysaccharide and sucrose, and β-glucan polysaccharide (1.0% (w/w) sol) and WPI, may have produced the larger than expected increase in sol viscosity observed.

The combination of β-glucan gum and WPI resulted in a slight increase in the thermal stability of whey proteins. Sols of 10% (w/w) WPI at pH 3 and 7, exhibited 2 and 3°C increases, respectively, in the temperature of denaturation with the addition of 0.5% (w/w) β-glucan gum. A significant increase ($p \leq 0.05$) in

the size of the endothermic peaks for both solutions, indicating increased energy input required for denaturation, was also recorded using differential scanning calorimetry (DSC). The physical presence of β-glucan polysaccharides may form a loose network, which physically inhibits the denaturation and aggregation of whey proteins. By increasing the gum to protein ratio, heat stability may further be increased. Increasing heat stability will aid in preservation of the native protein structure and functionality during processes such as pasteurization.

The addition of 0.5, 1.0, 3.0 or 5.0% (w/w) WPI to a 0.25% (w/w) β-glucan gum sol produced single-phase systems, where the concentration of the incompatible biopolymers was found to be below the phase separation threshold. Further increases in the concentration of β-glucan gum or WPI resulted in phase separation. Compositional analysis of phases was inconclusive due to the difficulty in separation of the relatively viscous top phases. Further detailed determination of phase composition would define the phase separation boundary for stable combinations of β-glucan and WPI. This information would be important in the formulation of stable food and beverage products.

The viscosity of β-glucan gum sols, at pH 3, was reduced significantly ($p \leq 0.05$) upon heat treatment at 75 and 95°C for 30 min. Sols at pH 5, 7 and 9, undergoing the same time/temperature treatments, did not exhibit the same decrease in viscosity. The combination of low pH and elevated temperature results in the acid-catalyzed hydrolysis of the β-D-glycosidic linkages of the β-glucan polymer and reduction of molecular weight (MW). Decreases in polymer MW result in the reduction of sol viscosity. Elevated processing temperatures,

such as those applied in pasteurization and hot-filling, may reduce the viscosity of acidic food and beverage products containing β-glucan. This could result in undesirable changes in β-glucan functionality and, ultimately, reduced consumer acceptability. It is, therefore, important to adjust food and beverage formulations or processing parameters to minimize such changes in functionality.

Orange-flavored functional beverages containing 0.3, 0.5 or 0.7% (w/w) β-glucan gum or pectin were successfully produced. Trained sensory panel results indicate that increasing concentrations of β-glucan gum or pectin did not result in suppression of beverage aroma. No significant changes ($p>0.05$) in beverage sweetness, due to the type of hydrocolloid or concentration, were found. Sourness, however, was shown to be slightly higher in pectin beverages than β-glucan beverages. The beverage highest in viscosity (0.7% (w/w) β-glucan gum) was found to be significantly ($p\leq0.05$) less sour than the lowest viscosity beverage (0.3% (w/w) pectin). The acidic nature of pectin, originating from lime peels, may have caused the slight increase in sourness observed. Whereas β-glucan gum, obtained from barley by alkali extraction and producing slightly alkaline, more viscous sols, may have reduced beverage sourness.

Viscosity of beverages with hydrocolloid concentrations ≥0.5% (w/w) was also found to differ significantly according to the trained sensory panel. Less β-glucan gum would be required to produce beverages of equal viscosity when compared to pectin. According to Burkus and Temelli (1998), β-glucan extraction conditions could be altered to produce low-viscosity β-glucan gum. This would allow the addition of more β-glucan soluble fibre into functional beverages

without undesired increases in viscosity. The higher fibre claims would be much more appealing to consumers, considering the potential health claims that may soon be ascribed to functional beverage products. The effect of MW reduction on the efficacy of the health-enhancing properties of β-glucan, however, is not known.

Consumer evaluation of 0.5% (w/w) β-glucan gum and pectin beverages determined that both beverages were equally acceptable ($p>0.05$). Analysis of beverages over 12 weeks of refrigerated storage indicated that beverage pH, microbiology and viscosity remained stable. In general, β-glucan beverage 'L', 'a' and 'b' colorimeter values decreased significantly ($p\leq0.05$) during the first week. These values then stabilized, showing no further significant changes ($p>0.05$) during storage. These changes in color are assumed to be due to the precipitation of insoluble material, temporarily stabilized within the beverages. Increase in β-glucan gum concentration resulted in a significant ($p\leq0.05$) increase in beverage whiteness, or 'L' value, and increase ($p\leq0.05$) in cloud, as measured by absorbance at 660 nm. β-Glucan beverage cloudiness decreased significantly ($p\leq0.05$) during the first three weeks of storage and then stabilized thereafter ($p>0.05$). These changes are also thought to be due to the temporary stabilization of insoluble material by β-glucan gum. No significant changes in the cloudiness of pectin beverages, during storage or due to hydrocolloid concentration, were observed ($p>0.05$).

Orange-flavored functional beverages, containing 0.5% (w/w) β-glucan gum and 0, 0.5, 1.0 or 1.5% (w/w) WPI, were also successfully produced. Trained

sensory panel results indicate that WPI addition significantly increased β-glucan gum beverage cloudiness ($p \leq 0.05$), increased sweetness slightly ($p > 0.05$), reduced sourness ($p \leq 0.05$), decreased orange-flavor intensity ($p \leq 0.05$), and significantly increased beverage viscosity ($p \leq 0.05$). The addition of WPI also imparted beverages with a specific whey flavor.

Consumer evaluation of the β-glucan/WPI beverages indicated that all of the beverage formulations were acceptable. However, acceptability scores for appearance, sourness, orange flavor, thickness and overall acceptance, decreased slightly with WPI addition, however, not significantly ($p > 0.05$). The trend also suggests that consumers prefer the sweeter taste of the WPI beverages, which scored slightly higher sweetness intensity scores in the trained panel evaluation. Consumer acceptability scores also suggest that a more sour beverage, such as the 0.5% (w/w) β-glucan beverage may be preferred over those with added WPI. The WPI beverages could, therefore, be formulated with increased levels of acid ingredients to improve β-glucan/WPI beverage sourness.

WPI addition significantly increased beverage cloudiness ($p \leq 0.05$), as indicated by the increased absorbance at 660 nm and increase in beverage whiteness ('L' value). Cloud loss was significant ($p \leq 0.05$) during the first 2 weeks of storage and then stabilized thereafter ($p > 0.05$). Beverages higher in WPI showed the greatest cloud loss and the beverage without WPI demonstrated the least decrease. The 'L' and 'b' colorimeter values remained stable throughout storage. Beverage 'a' values, however, continued to decrease slightly. This decrease became significant ($p \leq 0.05$) after the eighth week of refrigerated storage

in some beverages. Beverage viscosity and pH remained stable. Beverage pH was significantly increased ($p \leq 0.05$) with WPI addition. This is in agreement with the results of the trained panel, which indicated a reduction in beverage sourness with WPI addition, and the decreased sourness produced a slight decrease in consumer sourness acceptability scores for β-glucan/WPI beverages. This increase in pH and, therefore, reduced effectiveness of pH/pasteurization combination, may be responsible for the increase in microbial load seen in beverages with WPI added.

In this research, two beverage formulations incorporating barley β-glucan gum were developed and the final products were determined to be acceptable to consumers. These functional beverages show potential for commercialization. However, currently the main hindrance for the utilization of barley β-glucan as a nutraceutical and/or functional ingredient in food and beverage products is its high cost due to the high cost of extraction (Burkus, 1996). Development of a total fractionation procedure for barley and, optimization of protein-, starch- and lipid-rich fractions also produced during β-glucan isolation, would help reduce costs. Once such a process is developed, utilization of fractions other than β-glucan could be further developed, increasing the value of barley and making the extraction process more economically feasible. Until then, production of a β-glucan functional beverage is too costly.

Clinical investigation of the health enhancing properties of barley β-glucan and products enriched in β-glucan would further support any claims associated with the use of β-glucan in functional foods and beverages or

nutraceuticals. This will aid in the labeling of such products, once permitted in Canada, as expected in the future.

The effect of polysaccharide MW on health properties should also be determined. If reduced MW β-glucan has the same effects as high MW β-glucan, larger amounts could be used in food and beverage formulations without greatly altering functional properties. This would give products a higher soluble fibre content and, therefore, be more desirable for consumers.

Further characterization of barley β-glucan functional properties and examination of ingredient interactions are required to aid in the development of barley β-glucan enriched products. Development of an "instantized" or fast hydrating β-glucan gum would also improve its usage, by accelerating production time and allowing its incorporation into powdered beverage mixes. Such developments could result in increased barley β-glucan gum production, generating consumer demand, and eventually consumer "need" for barley β-glucan products (Pszczola, 1999). Thus, there are many possibilities in the rapidly developing functional food and beverage, and nutraceutical market for barley β-glucan. It is expected that β-glucan will soon be found on ingredient labels, enhancing product nutritional and functional properties.

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